UNIVERSITY OF BRISTOL

# THE ANNUAL REPORT

OF THE

## Agricultural and Horticultural Research Station

*(THE NATIONAL FRUIT AND CIDER INSTITUTE)*

## LONG ASHTON, BRISTOL

## 1948

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| | J. A. H. TOLHURST, B.Sc. (Agric. Chem.) (Hons.) (Reading). |
| | W. A. FORSTER, B.Sc. (Hons.) (Dunelm), Agricultural Research Council Plant Nutrition Scheme. (Appointed 1/1/48). |
| *Chemistry ; Insecticides & Fungicides :* | H. MARTIN, D.Sc. (Lond.), A.R.C.S., F.R.I.C. |
| *Bio-Chemistry :* | A. POLLARD, M.Sc. (Lond.), Ph.D. (Manchester). |
| | Miss. M. E. KIESER, B.Sc. (Hons.) (Bristol), A.R.I.C. |
| | Miss A. M. STONE, B.Sc. (Hons.) (Bristol). (Appointed 1/9/48). |
| *Organic Chemistry :* | D. WOODCOCK, M.Sc., Ph.D. (Dunelm), F.R.I.C. |
| | A. H. WILLIAMS, M.A., B.Sc. (Oxon.), Agricultural Research Council Plant Nutrition Scheme. |
| *Physical Chemistry :* | W. D. E. THOMAS, B.Sc. (Hons.), Ph.D. (Wales), A.R.I.C. |
| *Entomology :* | H. G. H. KEARNS, B.Sc. (Lond.), Ph.D. (Cantab.), Dip. Agric. |
| | S. H. BENNETT, B.Sc. (Hons.) (Bristol). |
| | N. G. MORGAN, B.Sc. (Hons.) (Bristol). |
| | A. STRINGER, B.Sc. (Hons.), A.R.C.S. (Lond.), Agricultural Research Council Insecticidal Bio-Assay Scheme. |
| *Mycology :* | R. W. MARSH, M.A. (Cantab.). |
| | S. H. CROWDY, B.Sc. (Hons.), A.R.C.S. (Lond.). |
| *Plant Physiology :* | E. J. HEWITT, B.Sc. (Hons.) (Lond.), Ph.D. (Bristol), A.K.C. |
| | W. PLANT, B.Sc. (Hort.), Ph.D. (Reading), N.D.H., Agricultural Research Council Plant Nutrition Scheme. |
| | E. W. JONES, M.Sc. (Wales), Agricultural Research Council Plant Nutrition Scheme. |
| *Pomology :* | G. T. SPINKS, M.A., Dip. Agric. (Cantab.). |
| | L. C. LUCKWILL, B.Sc. (Hons.), Ph. D. (Bristol). |
| | E. W. HOBBIS, B.Sc. (Bristol), N.D.H., Plantations Officer. |
| | W. R. STANTON, B.Sc. (Hort.), (Reading), Cider Orcharding. (Resigned 29/2/48). |
| *Cider :* | B. T. P. BARKER, M.A. (Cantab.) (Part-time). |
| | S. W. CHALLINOR, B.Sc. (Hons.), Ph.D. (Birm.), D.Sc. (Edin.), F.R.I.C. |
| | L. F. BURROUGHS, B.Sc. (Hons.) (Reading), A.R.I.C. |
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| | Mrs. D. P. JAMES, B.Sc. (Bristol). |
| | Miss M. STURDY, B.Sc. (H. & S.S.) (Lond.), Instructress. |

# ASSISTANT STAFF.

| | |
|---|---|
| *Plant Physiology :* | Miss O. M. Woodhouse, B.Sc. (Hort.) (Lond.), Recorder. (Agric. Research Council Plant Nutrition Scheme). (Appointed 1/11/48). |
| | Miss A. Haskins, Assistant Recorder. (Resigned 21/2/48). |
| *Pomology :* | S. H. Legge, Marketing Officer and Recorder. |
| | G. E. Clothier, Station Guide and Meteorological Recorder. |
| | E. Umpleby, Propagator and Recorder. (Resigned 30/4/48). |
| | J. W. Bultitude, N.D.H., Propagator and Recorder. (Appointed 1/6/48). |
| | E. Catlow, Recorder. |
| | R. M. Jarrett, Recorder. |
| | Miss R. M. Stickley, B.Sc. (Hort.) (Lond.), Recorder. (Appointed 1/3/48). |
| | H. Locke, Horticultural Assistant. |
| *Laboratories :* | F. E. Maltby, Chief Laboratory Steward and Research Assistant. |
| | A. H. Wilkins, Research Assistant (Entomology). |
| | G. H. Jones, Research Assistant (Chemistry and Photography). |
| | R. F. Batt, Research Assistant (Chemistry : Insecticides and Fungicides). |
| | W. J. Redmond, Analyst. (Agricultural Research Council Plant Nutrition Scheme). |
| | Miss M. D. Atherton, Assistant (Fruit and Vegetable Preservation). |
| | Mrs. E. J. Bunker, Assistant (Fruit and Vegetable Preservation). (Resigned 30/11/48). |
| | Miss B. L. Febrey, Assistant (Pomology). |
| | A. H. Fielding, Assistant (Mycology). |
| | M. A. Wright, Assistant (A.R.C. Plant Nutrition Scheme). |
| *Plantations :* | A. J. Hurdle, Plantations Foreman. |
| | Miss C. E. C. Clarke, Recorder. |
| | Miss E. I. M. Prescott, Assistant Recorder. (Resigned 31/10/48). |
| | Mrs. M. R. Lewis, Assistant Recorder. |
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| | J. H. Llewellyn, Technical Assistant. |
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| *Office :* | Mrs. A. I. Edbrooke, Cashier. |
| | Miss A. M. Evans, Director's Secretary. |
| | R. A. Merchant, Chief Clerk, Accounts. |
| | D. H. Pittman, Senior Clerk. |
| | J. Packer, Ledger Clerk. |
| | Miss E. M. Matthews, Clerical Assistant. |
| | Miss I. A. Pearce, Clerical Assistant. (Resigned 5/6/48). |
| | Miss V. E. R. Alsop, Clerical Assistant. |
| | Miss I. Trickey, Clerical Assistant. (Resigned 3/4/48). |
| | Miss I. E. Taylor, Library Clerk. |
| | Miss M. Anstey, Clerical Assistant. (Appointed 21/6/48). |
| | Miss J. I. Curtis, Telephonist. |
| *Secretary :* | A. L. Leggett, A.S.A.A. |

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# INTRODUCTION.

By T. WALLACE, C.B.E., M.C., D.Sc., F.R.I.C.

1948 has been a year of steady progress in which it has been possible to develop the work in the various sections along the lines visualised for the immediate post-war period. With the more complete establishment of the National Agricultural Advisory Service fewer advisory enquiries were addressed to the Institute though a very close liaison has been maintained between the staffs of the two organisations. This has been achieved in various ways. Thus local contacts have been maintained by providing library and other facilities at the Institute for the National Agricultural Advisory Service provincial staff and by inviting them to participate in staff discussions. The Institute has also functioned as the headquarters of the south-west branch of the Horticultural Education Association and has been a meeting place for N.A.A.S. and local County Council officers to discuss problems of mutual interest with members of our staff. In addition, lecturers have been provided for N.A.A.S. Conferences in different parts of the country. These arrangements should do much to ensure that the practical results obtained in investigations at the Institute are made available to growers without undue delay.

The Cider Advisory Committee and its sub-committees have again been active during the year, particularly with a view to stimulating interest in the production of cider fruit. A further orcharding conference was held at Totnes in September where papers were read and practical orcharding demonstrations given. At the suggestion of the Committee, Provincial Officers of the N.A.A.S. called a meeting of a Joint Cider Orcharding Committee for Devon, Dorset and Somerset to discuss problems of cider apple production, at which resolutions were passed which aimed at improving orchard conditions, obtaining better seasonal estimates of cider fruit crops and suggesting means of fixing cider fruit prices. Following the publication of the list of recommended cider varieties for post-war planting, the Committee has considered the possibility of establishing demonstration orchards of both bush and standard trees, not only in the recognised cider orcharding districts of the West Midlands and the South West but also in East Anglia and the South East. It is hoped that a scheme for these trials will be ready early in 1949.

The new arrangements for work on Cider and Fruit Products came into operation in March and programmes of research were approved by the new Agricultural Research Council Committee in June. Work is proceeding smoothly under the scheme but must of necessity remain on a small scale until the proposed new building for small scale factory experiments is available.

During the year the Institute suffered a great loss by the death of Mr. John Pullin, who had been a member of the Institute since 1912 and a Governor since 1930. Mr. Pullin had also served on the Managing Committee of the Institute and on the Agricultural Committee of the University since 1930.

Valuable as his services had been in those connections, his association with the Institute was still closer and more intimate. Selected by the Gloucestershire County Council to act as Instructor in Cidermaking in the County, he was sent to Long Ashton before the first world war for a course of training in the subject. Thus began a personal link with the Station and its staff which continued to grow stronger with the passing years. In his own county and over a wider area he gave his whole-hearted support to its work and welfare. It was thus with a special degree of satisfaction to himself

and the Institute that at the Annual Meeting of Members in 1940 he was presented with the McCreath Cup, awarded to the exhibitor obtaining the highest aggregate awards in Open Cider Competitions during the period 1937-1939.

Certain changes occurred in the membership of the Institute and University Committees which call for mention.

Mr. B. Worrall, who had been a member of the Finance and Business Sub-Committee since 1941, resigned on leaving the district to live in Cornwall. Dr. H. V. Taylor, a very old friend of many members of the Institute, retired from his official post in the Ministry of Agriculture, as Senior Advisory Officer in the N.A.A.S., and expressed his desire to resign from the Institute Managing Committee and the Finance and Business Sub-Committee. Dr. Taylor had served on these bodies since 1928 and has rendered much valuable service to the Station. His wise counsel and his ready assistance in dealing with official bodies will be greatly missed. Our best thanks are due to both Dr. Taylor and Mr. Worrall, and members will wish us to express here their appreciation of their services to the Institute and to wish them both many years of happiness in their retirement. We shall hope to see them in the future at Institute gatherings.

Another noteworthy change occurred in the retirement of Mr. J. H. Keeler, who had been the Station auditor since 1919. Mr. Keeler had given much help with financial problems throughout his professional association with the Station. On his retirement the Agricultural Committee invited him to serve on the Finance and Business Sub-Committee and we are happy to report that he will continue to help in this new capacity.

It was also possible during the year to arrange for Mr. T. N. Hoblyn, assistant to the Director of the East Malling Research Station, to serve on the Finance and Business Sub-Committee. As the Long Ashton Director is already a member of the corresponding Committee of the East Malling Station, the new arrangement should ensure a very close co-operation between the executive committees of the two Stations.

Since 1948 was the first year of working the revised membership scheme, it is of interest to note that the numbers of members and associates in December 1947 and 1948 respectively were 563 and 550. It would thus appear that the curtailment of advisory privileges of membership which became necessary on the establishment of the National Agricultural Advisory Service has resulted in only a small loss of members.

At the annual meeting of members in July it was decided to establish a scholarship to commemorate the outstanding services rendered to the Institute by Mr. John Wootton, Mr. Eldred Walker and Mr. John Ettle. The Managing Committee was asked to devise a suitable scheme and to put it into operation for the 1949 cider making season.

An appropriate scheme has been evolved which has received the financial support of the Agricultural Committee. It is hoped that a suitable student will be found to enable the scheme to be initiated in October, 1949.

The details of the Memorial are as follows :

*Memorial Studentship to Commemorate the Services Rendered to the Institute by Mr. John H. Wootton, Mr. Eldred G. F. Walker and Mr. John Ettle.*

(1) The Memorial shall take the form of working studentships tenable at Long Ashton in cider making and/or cider orcharding, and shall be of six months' duration.

(2) One studentship shall be available for award annually.

(3) It shall be open to the sons of farmers and farm workers in the counties of Devon, Gloucester, Hereford, Monmouth, Somerset and Worcester.

(4) Awards will be made by the Managing Committee of the Institute from nominations submitted by the County Councils.

(5) Students elected will receive free tuition in cider making and/or cider orcharding at Long Ashton. In addition, a subsistence allowance, at the rate of £100 for the full six months' period of the course, will be made.

(6) Nominations should be submitted by County Councils by the 31st March each year.

(7) The name of the selected student for each year will be announced at the Annual General Meeting of Members and Associates of the Institute.

**Staff.**

Changes in the scientific staff were relatively few during the year.

Mr. W. A. Forster was appointed to succeed Mr. Colin F. Mills as research assistant (chemistry) under the special Agricultural Research Council grant for mineral deficiencies investigations and took up his duties on January 1st, 1948. Mr. Forster graduated at King's College, University of Durham, in 1945, and worked during 1946 and 1947 as a research chemist in the Shell Chemical Research Laboratories at Thornton Research Centre, Chester.

Miss Audrey Stone was appointed to succeed Miss Joan D. Bryan as research assistant (chemistry) in the Fruit Products Section. She commenced her duties on September 1st, 1948. Miss Stone graduated in 1947 at the University of Bristol and was employed by Messrs. H. W. Carter & Co. Ltd., of Bristol, from October, 1947, to July, 1948.

Efforts were also made to fill three other vacancies on the scientific staff, two in Pomology and one in Plant Physiology (for work in the Plant Pathology team) but no suitably qualified candidates were found. These posts will be re-advertised during 1949.

Mr. H. Gleen, of the Agricultural Research Council team of workers on soil metabolism, continued to work at the Station throughout 1948.

Mrs. M. B. Tuke, a member of the National Agricultural Advisory Service, has been studying the technique of sand cultures since 13.12.48, with special reference to experiments with bulbs, which she will later continue at Kirton, Lincs.

Appointments to the assistant staff were as follows :

Miss R. M. Stickley received her training at Studley College and graduated B.Sc. (Hort.) at London University in 1947. She was appointed recorder in Pomology (Cider) on 1.3.48.

Miss O. M. Woodhouse, who was a student at Nottingham University College, and graduated B.Sc. (Hort.) at London University in June, 1948, was appointed recorder under the Agricultural Research Council Plant Nutrition Scheme on 1.11.48.

Mr. J. W. Bultitude was appointed Propagator on 1.6.48 : he had previously been a student at Wisley, served in the R.A.F. during the war and then returned to Wisley as a Pomologist for the National Fruit Trials.

The following resigned their posts during the year :

Mr. W. R. Stanton, Research Assistant (Cider Pomology) on 29.2.48.

Mr. E. Umpleby, Propagator and Recorder since 1925, from 30.4.48, to take up a commercial post in Devon.

Mrs. E. J. Bunker, Research Assistant (Fruit and Vegetable Preservation) from 30.11.48.

Miss A. Haskins, Assistant Recorder (Physiology) from 21.3.48.

Miss I. A. Pearce, Clerical Assistant, from 5.6.48.

Miss I. Trickey, Clerical Assistant, from 3.4.48.

During the year some important visits abroad were made.

At the invitation of the President of the John Hopkins University, Baltimore, U.S.A., the Director attended a Conference at that University in April, to discuss the organisation of research and other activities to be financed by a fund to be known as the McCullom-Pratt Fund, to be applied in extending knowledge of nutrition in relation to Plants, Animals and Man. After the Conference a tour was made of Research Centres and Colleges and some fruit growing areas in the eastern states during the course of which a number of lectures were given on behalf of the Ministry of Agriculture and the Agricultural Research Council. Centres visited included the U.S. Department of Agriculture Research Centre, Beltsville ; Pennsylvania State College of Agriculture ; Cornell University ; New York Agricultural Research Station, Geneva ; Boyce Thompson Institute ; and Rutgers University. The main fruit areas visited are located in the States of Virginia, West Virginia and Pennsylvania. The visit necessitated an absence from Long Ashton from March 31st to May 13th.

Dr. H. Martin attended the 8th International Congress of Entomology, held at Stockholm from August 9th to 14th, 1948.

Dr. S. H. Challinor and Dr. A. Pollard were awarded travelling fellowships by the Ministry of Agriculture to visit Switzerland and France to study methods of cider and fruit juice manufacture in those countries. The first part of their tour was made in Switzerland as members of an organised tour of the National Association of Cider Makers, after which they proceeded to France to visit centres in Paris and the cider districts of Normandy. The main centres visited were : *Switzerland* : A Cider and Fruit Juice factory at Muttenz, near Basel ; the Wine Cellars of Messrs. August Egli and Co. at Zürich-Leimbach ; the Cider and Apply Juice factory of Messrs. Zweifel and Co., Zürich-Hongg ; the V.O.L.G. Co-operative Wine, Cider and Apply Juice Cellars at Winterthur ; the Mosterei und Obstverwertungs-genossenschaften at Märwil ; a Cider and Apple Juice factory at Affeltern ; the Research Station at Wädenswil ; the University at Zürich ; the Zürich Laboratories of the Unipektin Corporation. *France* : The Laboratories of the Union Nationale des Producteurs de Jus de Fruits ; the Laboratories of Prof. Lemoigne at the Institut Pasteur ; the Cider and Apple Juice factory of MM. Caumont Fils, at Wissous ; the factory of MM. Charmat, near Paris ; and the factory of Les Cidreries du Calvados at Livarot, Normandy ; the Station des Recherches Cidricoles, Agricoles et Laitieres at Rennes.

Dr. W. D. E. Thomas attended a course in radio-active element technique during the week beginning 18th October, 1948, organised by the Medical Research Council and held at the Council's Radiotherapeutic Research Unit Headquarters at Hammersmith.

The Director delivered the Royal Horticultural Society's Masters Memorial Lectures on June 8th and October 19th, 1948. The subject chosen was " Nutrition problems of horticultural plants, with special reference to trace elements."

Dr. S. W. Challinor was awarded the degree of Doctor of Science of Edinburgh University in July, 1948, for a thesis entitled " Studies in microbiological chemistry."

During the year the scientific staff were assimilated into the new grading system based on the " White Paper " grades for the Scientific Civil Service with effect from January 1st, 1946. The laboratory assistant staff were also graded with effect from January 1st, 1947, final assimilation being completed in January, 1949. The assimilation of the administrative and clerical staffs remains to be effected.

The annual Christmas Staff Party was held on December 20th and was attended by 212 members of staff and their friends. The programme included games and a pantomime, produced and played by members of the assistant staff, these events being followed by supper and dancing. The evening was, as usual, a great success and the arrangements made were appreciated by all. Thanks are due to all who contributed to the success of the gathering and particularly to those responsible for the pantomime and the arrangements for and serving of the refreshments.

**Buildings and Land.**

The new Biology Building mentioned in the 1947 Report was completed early in March and occupied on March 16th. This additional accommodation has proved of great value in developing the research programme in plant pathology and full use has been made of it by the entomologists and mycologists. One of the rooms has been fitted as a chemical laboratory temporarily to house some of the staff of the Plant Nutrition section and another has been occupied by two members of the Pomological staff. It is hoped that the early provision of further accommodation will free these rooms for Pathology.

The new Transformer House was also completed during the early months of 1948 and the electric supply connected to it.

The contract for the external painting of all the main buildings of the Station was placed early in 1948 and the work carried out by Messrs. William Cowlin & Sons, Bristol, during the period March to August. The completion of this work has removed a great anxiety for the state of the property.

Plans for the small building for the preparation of pots and sand for pot culture experiments were completed and work began on its erection in September. It is hoped that the fitted building will be ready for use early in 1949.

Further progress has been made with the plans of the special building for the Cider and Fruit Products Sections. The actual plans are now in final form, so far as the staff are concerned, and have been forwarded by the architect to the quantity surveyor. The next step will be to obtain the approval of the Agricultural Research Council Committee and the Ministry of Agriculture to the plans prior to sending out to tender. It is hoped that this last stage will be reached early in 1949.

Consideration has also been given by the Finance and Business Committee to future building requirements of the Station and decisions have been made on these and on the priorities in regard to them. The main requirements which have been agreed upon by the Committee are as follows : New Laboratory Block ; Special Purpose Greenhouses for Plant Nutrition, Pomology. Entomology and Mycology, combined with special purpose small rooms providing facilities for controlled environments for growing plants, for low temperature experiments and for radio-active tracer investigations ; Canteen

and Staff Common Room; Offices; Farm Buildings and Fruit Stores—including Low Temperature and Gas Store chambers.

In connexion with these proposals a site plan has been prepared, at the suggestion of Sir John Fryer, for submission to the Agricultural Research Council and the Ministry of Agriculture.

The necessary re-painting of the large plant nutrition greenhouse was also carried out satisfactorily during the summer months and the condition of this structure has been much improved.

Good progress was also made in the cider house with cleaning, repair work and painting, all of which had of necessity fallen greatly in arrears during the war years. Three new Ebon-lined concrete vats, total capacity 4,200 gallons, were also fitted in the cellar to replace defective wooden storage vats.

Following a survey of the fire protection equipment by the National Fire Service, new equipment was installed in various buildings to make good defects in the existing arrangements. Plans were also drawn up for a special building to be erected in the orchard near the main laboratories for the storage of inflammable chemicals for which greatly increased storage space has become necessary due to the large increase in staff which occurred during the war.

In 1939, two grass tennis courts adjoining the main laboratories were available to the staff for recreation purposes but during the war the lawns deteriorated and the surrounding wire fence became dilapidated. During 1948, it was decided to restore the area for tennis and, in view of the increased staff, to replace one of the grass courts by a hard court. Arrangements on this basis are being made and it is hoped that both courts will be ready for play early in 1949.

In August the Director received an offer of an old stone cider mill installed at Bollingham, Herefordshire, from Alderman George Bray, C.B.E., Chairman of the County Estates and Small Holdings Committee, through the medium of Mr. Greville Phillips, County Land Agent. The offer was gladly accepted and the mill was transferred to Long Ashton in September, where it will be assembled on a suitable site after the completion of the new Pilot Factory. The mill will be of interest as a museum piece and it is also hoped that it may be of use in comparing old and new pressing methods. The thanks of the Institute are due to Alderman Bray and his Committee and to Mr. Greville Phillips for this interesting gift.

During the year it has been necessary to carry out considerable repair work on the boundary fences of the fruit plantations. The problem of replacing some sections of the fences is an urgent one and the position is difficult due to the scarcity of suitable materials.

Further progress has been made in implementing the post-war planting programme. The remainder of the new Plot 24—3 acres—was planted with dessert plums during the autumn. These trees will be mainly for the use of the Pathologists.

A section of the existing plum plot—Plot 18—was cleared of trees during the season and the remainder of the plot will be cleared in 1949. It is proposed to use this site for a combined cover crop manurial trial on apples. The cleared areas will be suitably cropped and cleaned during the next two years in preparation for planting. Preparation of the site for the new bush cider orchard has also been commenced by the felling of hedgerow trees.

Two other small plots were planted. One consisted of 232 trees of the apple variety Lord Lambourne, occupying $\frac{1}{3}$rd of an acre, which are to be

used in investigations of the troubles affecting this variety. The other comprised commercial and cider varieties of apples, worked on M.II rootstock, and grown as dwarf pyramids, planted at 9′ × 6′. The area planted was ½ an acre and will be extended when trees are available to 3 acres. The trees are to be used for pathological investigations.

In the nursery good progress has been made in building up supplies of apple rootstocks to meet the needs for trees for the new Station plots and also for the proposed cider trial orchards.

Five of the selected sweet cherry seedlings raised at the John Innes Horticultural Institution—Merton Heart, Merton Bounty, Merton Premier, Merton Favourite and Merton Bigarreau—are being propagated at Long Ashton to supply scion material to the nursery trade and to cherry growers. A small amount of material will be available from the end of the 1948 season. One of the East Malling seedling varieties of raspberries, 51/84, is being propagated for that Station to assist in keeping the variety virus free.

Under the Seedling Distribution Scheme of the Station, varieties of apples, pears, plums and black currants have been supplied to various research and educational centres in England and also to Denmark, Eire, France, Guernsey, Holland, Kenya, Norway, Sweden, Switzerland and U.S.A. Many fruit growers and nurserymen have also been supplied. Varieties most in demand are the pears, Bristol Cross and Cheltenham Cross, the late dessert plums, Thames Cross and Severn Cross, and the three black currants, Mendip Cross, Cotswold Cross and Malvern Cross.

On the whole, fruit crops, with the exception of plums, were satisfactory. Soft fruit yields, particularly black currants, which form a large proportion of the acreage, were heavy. The actual weight of black currants harvested was 14½ tons. Apple yields varied according to varieties, Worcester, Allington, Laxton's Superb, Bramley, Edward VII and Crawley Beauty all giving heavy crops whilst Cox's Orange Pippin was notable among the light cropping sorts. The cider varieties yielded 38 tons, good crops being carried by the varieties Stoke Red and Sweet Alford on the bush trees.

**Research Activities.**

In the investigations on mineral deficiencies of farm crops, field plots were established to study soil acidity factors and the possible relationship of "Whiptail" in cauliflower to molybdenum deficiency. The first season's experience has shown the practical difficulties of conducting acidity experiments on very poor soils. The results suggest differences in problems associated with acid conditions on different soils and show the need for detailed studies of the effects of "liming" treatments on a variety of soils. No definite conclusions are possible so far from the "Whiptail" trials and further experiments have been planned.

Problems relating to soil acidity were also continued in sand culture experiments. Here the main points under investigation were the effects of calcium deficiency and excesses of manganese and aluminium on a wide range of crop plants, the interactions of the above factors, particularly Ca/Mn and Mn/Al and the interactions between Mo/Mn. In the survey experiments covering a variety of crops, the differential effects of excesses of manganese and aluminium were clearly shown. Thus brassicae as a whole seem very sensitive to manganese toxicity, whilst sugar beet, celery, leek and carrot are easily damaged by excess aluminium. When both elements were present in excess this sometimes accentuated the effects of one of them, e.g. manganese

in swedes, and sometimes the reverse was shown, e.g. aluminium toxicity effects were reduced by excess manganese in carrot etc. Celery and potato were shown to be particularly sensitive to calcium deficiency.

The relationships of Mn/Mo appear to be complicated. Thus with cauliflower the addition of molybdenum appeared to accentuate damage from excess manganese in young plants but to be beneficial to mature plants. Deficiency of either of these elements results in accumulation of nitrate in the leaves, but with molybdenum deficiency amino acid content was low and with manganese deficiency high.

A comprehensive experiment was carried out in sand culture on potatoes to examine in detail the effects of potassium/iron relations. K and Fe were each applied at four levels in all possible combinations in the presence and absence of calcium carbonate. Detailed analysis for chlorophyll, extractable iron, potassium and phosphorus were made in addition to making growth records. The statistical analysis of the results is not yet complete but one point was obvious—that the growth of plants receiving low levels of iron was greatly improved by high levels of potassium. The reverse effect of iron on potassium, however, was not clearly shown.

Experiments were also continued on the inducing of iron deficiency by various metals. The effects of the metals, as reported for previous experiments, were generally twofold—direct damage and induced iron deficiency. The effects for the latter were as follows :

| | |
|---|---|
| Severe : | $Co^{++}$, $Cd^{++}$, $Cu^{++}$, $CrO_4''$, $(Zn^{++})$ |
| Moderate : | $Zn^{++}$, $VO_3'$, $Ni^{++}$, $Cr^{+++}$, $MoO_4''$ |
| Slight : | $Ti^{+++}$, $AsO_4'''$, $Mn^{+++}$, $Pb^{++}$ |
| Trace or Nil : | $Sn^{++}$, $Ce^{++++}$, $WO_4'$, $Tl^{++}$ |

Some of the direct damage effects were particularly severe and characteristic, especially those from nickel and cobalt.

Molybdenum appeared to increase iron deficiency effects in sugar beet induced by $Cr^{+++}$, $Mn^{++}$, $Cu^{++}$ or $Zn^{++}$, which is contrary to the results of Millikan with flax in water culture. Chlorosis was generally delayed by the substitution of urea for nitrate as a source of nitrogen but growth was poorer.

Further experiments were made to improve points of technique in relation to water and sand purification, container linings and purification of nutrient reagents. Manganese level in rain water has been reduced to 0·002 p.p.m. Mn by means of a chain of " Permutit " ion exchange units.

A fine-grained sand from Loch Aline, Scotland, was found to be suitable for iron deficiency work but is liable to algal growth. Doulton glazed pots were suitable for experiments on manganese and boron deficiency but not for iron deficiency. A method, using *Aspergillus niger*, is being used to check nutrient reagents for Mo, Cu and Zn content.

In investigations to determine the effects of mineral nutrients on the organic constituents of plants a preliminary examination has been made of the tannin complex in the bark of apple trees. Chromatographic separation on magnesium oxide suggests the tannin fraction to be a complex mixture showing a continuous gradation of properties. The method of paper chromatography has also been applied to the examination of amino acids in leaves of plants deficient in Mn and Mo respectively (see above).

In further work on chemical tissue tests, the use of the Waring Blendor as a means of extracting " readily soluble " nutrients has been examined and the results compared with the usual Morgan reagent extraction methods.

With the former it is necessary to clear the extracts with a special absorbent carbon and for this purpose Darco G 60 has proved suitable. The Waring Blendor gives higher (though fairly parallel) results than the Morgan's reagent extraction method, but is only suitable for laboratory determinations.

Tissue test methods have been extensively applied to a variety of field crops with a wide range of nutrient status and continue to give satisfactory results.

An experiment was carried out at three centres on barley crops in an attempt to correlate chemical data on plant tissues at various growth stages with crop yields. The results are undergoing statistical analysis.

Methods have been devised for the use of *Aspergillus niger* for the bio-assay of Mg, Cu, Zn, Mn and Mo in soils. Tests of the method on a range of soils have given promising results.

A preliminary experiment was carried out with radio active phosphorus to follow the uptake of phosphate applied as a fertiliser. (The $P^{32}$ had been prepared by the irradiation of disodium hydrogen phosphate and it was shown that this method is unsuitable for plant nutrition experiments, as less than 50% of the $P^{32}$ remained in phosphate form after irradiation treatment).

In field plots on apples the production of magnesium deficiency from the prolonged use of an N.P.K. fertiliser treatment is now very evident. In the same trials on plots where N only has been given since 1935, shoot growth is restricted and the main problem is potassium deficiency.

On the bush cider apple plot results from differential cover crop treatments are now becoming apparent (after 3 seasons) and rootstock effect is also significant. Grass and clover treatments have produced significantly higher P and K contents of the leaves than an annual cover crop or clean cultivation treatments. Nitrogen content also tends to be lower on the grass and clover plots. Magnesium is low on all plots, particularly so where nitrogen content is low.

Magnesium content is significantly lower in trees on Malling No. 1 rootstock than in those on Seedling Crab. The varieties also show differences in regard to their reaction to deficiency conditions, Sweet Alford being outstanding in severity of effects due to deficiencies of Mg and Mn.

In other experiments on apples magnesium deficient trees have responded readily to foliage spray treatments and very spectacular effects from this treatment were obtained in an experiment on Laxton's Superb. Trials with a " Mapic " soil injector to introduce nutrients at various depths into deficient soils have not so far produced beneficial results but further trials are necessary to determine the best method of using this apparatus.

Experiments have been planned for 1949 to examine suspected instances of deficiencies of zinc and copper in apple trees on the R.H.S. Fruit Trial plots at Wisley. The symptoms shown are " little leaf " and " die-back."

It is proposed to make comprehensive studies of the effects of various grasses and legumes on soil structure and on the mobilisation and movement of nutrients in soils, and to this end special plots are being established both in the open and in apple plantations to test the results under these two conditions, the final aim being to develop desirable cultural systems for orchards.

Results to date have shown the beneficial effects of a grass cover on P.K. and Fe and the lowered N status which results, at least in the early years, from its use. A striking effect of this treatment in controlling K deficiency may be seen on plots (in conjunction with Somerset County Council) at Cannington.

In the black currant manurial experiments the low availability of nitrogen in sewage sludge/straw composts and farmyard manure continues to be clearly shown. Sewage sludge (not composted) has proved to be an excellent supplier of nitrogen.

Three varieties of potato, Majestic, Kerr's Pink and Red King, were grown on the rotation-manurial plots and showed several points of interest. The three varieties reacted differently to deficiencies and high manurial treatments. Majestic was least affected by severe deficiencies of K or N and less severe deficiency of Mg ; Kerr's Pink was very sensitive to deficiencies, giving very low yields, but it responded most to generous manuring, under which conditions it produced the highest yields. Potassium and nitrogen are the outstanding deficiencies on these plots and, provided N is applied, yields are generally correlated with K content of the plants. Where common salt is given *vice* muriate of potash, K deficiency was in evidence in 1948. K deficiency also occurred where N.P.K. plus Mg is applied, presumably due to the depressive effect of Mg on K, and where a compost of sewage sludge and straw is given, showing that the straw cannot make good the K deficiency of the sludge for a high K crop such as the potato. Very obvious effects in decreasing magnesium deficiency in potato haulm resulted from the use of magnesium sulphate sprays (2% Epsom Salts plus spreader), but tuber yields were not significantly increased.

In the pomological section the new plantation of apples, to study different systems of planting, has been successfully established. The planting systems using Cox Orange Pippin throughout, are :—bush trees on M II at 24′ × 24′ ; a similar plot with fillers on M IX at 12′ ; dwarf pyramids on M II at 8′ × 4′ ; cordons on M II at 8′ × 3′. A small number of trees on Winston and Superb are included in the plots as pollinators.

Trees of the apple variety Lord Lambourne, using scions taken from supposedly healthy trees and worked on rootstocks I, II, IV, VII, IX, XII and XVI, have been planted out from the nursery to follow their histories with reference to the various troubles that afflict this variety (i.e. rubbery wood, chat fruit, mottle etc.).

Investigations on growth substances, concerned with fruit set, parthenocarpy and fruit drop, have been continued. Using Lane's Prince Albert, it was possible to correlate waves of fruit dropping with the total hormone content of the seeds as determined by the tomato ovary test. It has been confirmed that the hormone responsible for the control of fruit drop originates in the endosperm tissue. Attempts have been made to concentrate the seed hormone by chromatographic methods. Its sensitivity to both acid and alkali hydrolysis has shown that it cannot be identical with either auxin A or indolyl-acetic acid.

The growth substances 2 : 4D, A N A and I A, have been tested for fruit set and fruit drop, and trials have been made with the former two to delay bud burst. No results of practical significance have so far been obtained.

In the plant breeding section an early dessert variety of good appearance and fair quality (slightly on the acid side) has been selected as worthy of further trial. Its season is definitely in advance of Worcester.

The pear variety Bristol Cross continues to give heavy crops of good quality and has recently been recommended by the National Fruit Trials Committee for further trial.

The black currant varieties, Mendip Cross, Cotswold Cross and Malvern Cross, again yielded heavy crops in 1948.

In field trials the entomologists have given special attention to the efficacy of automatic spraying methods for the control of red spider. It is concluded that the method is likely to give satisfactory results when used during the dormant and post-dormant periods, but during the full leaf period the coverage of the lower leaf surfaces is not likely to be adequate and is not as good as is obtained by manual methods.

D D T at ½ lb./100 gall. as a benzene-sulphite lye emulsion and added to 3% petroleum oil (Shell J D 2) applied at aphis hatch, gave complete control of *Aphis pomi* on apples.

Where D D T has been used, however, red spider has been higher than with D N C petroleum and woolly aphis has also shown an increase, particularly on the variety Laxton's Superb.

Bio-assay methods have been applied to problems of D D T and systemic insecticides. With D D T it was demonstrated that the insecticidal properties are dependent upon the combination of two chlorophenol groups with a trichloroethane group and that modification of these results in loss of toxicity. Results of tests on the chlorination of the various groups suggest that the spatial configuration of the molecule is of importance.

Investigations with the systemic insecticides, bis(2-fluoroethoxy methane), bis(dimethylamino)fluorophosphine oxide and some others, have shown them to act as fumigants on specific insects as well as systemics.

The manner in which the apple canker fungus *Nectria galligena* infects through leaf scars has been examined. Infection occurs in freshly exposed scars by spores which are sucked into the tracheids. Comparisons of subsequent development of the spores on the susceptible variety Beauty of Bath and the more resistant Bramley's Seedling showed that, whilst infection occurred readily in both sorts, in the former the infections spread into the surrounding tissues whereas in the latter they remained small and were confined to the immediate neighbourhood of the scars. Anatomical investigations have established that whilst the pathogen is generally readily checked in living tissues it spreads freely in the fibres. Field trials with growth substances have shown that beneficial effects from these were associated with their ability to stimulate the host rather than to check the pathogen.

Trials of winter spray treatments to control infections of Brown Rot (*Sclerotinia fructigena*) on mummied apple and plum fruits have shown the value of phenyl mercuric chloride and monocalcium arsenite for this purpose. Summer spraying treatments on plums with 1% lime sulphur and 0·2% of a phenyl mercuric chloride preparation also reduced the percentage of infected fruits.

A condition of plums, superficially resembling "rubbery wood" of the apple Lord Lambourne, has been observed on a number of varieties including Bountiful, Giant Prune and Thames Cross. The condition has been given the name "Stunted Plum" and may prove to be a Virus disease.

Tests with glyoxalidine against apple scab (*Venturia inaequalis*) and black currant Leaf Spot (*Pseudopeziza ribis*) showed that this material is a promising fungicide. With scab it gave better results than four lime sulphur sprays and against Leaf Spot it increased foliage retention from 26% on controls to 74%. Its use on black currants before picking time did not affect canning qualities, flavour or appearance of the fruit.

In cider investigations attention was given to problems of vintage quality in relation to the maturity of the fruit at pressing and detailed investigations were begun on three methods of fermenting juices, viz. (i) spontaneous, (ii)

dominant and (iii) pure fermentations. It has been shown that relatively stable juices may be obtained by the use of ion exchangers and that the increased stability is apparently largely due to the removal from the juice of mineral nutrients. The stabilised juices were rendered active again by the addition of the elements N, P, Mg and Ca. The method should be valuable in determining the mineral requirements of yeasts and other organisms concerned in fermentation. Comprehensive investigations have been started on the chemical constituents of cider. So far they have related to nitrogen, total acidity, "tannins," alcohols, volatile substances other than alcohols, esters and aldehydes.

It has been shown that the disorder of ciders known as "Cider Sickness" is probably caused not by one organism only but by a group of closely related organisms, the position being analogous to acetification where a group of acetic bacteria are concerned.

The fruit products section has now commenced on its post-war programme after completing various lines of work undertaken during the war period. The programme on apple juice includes factors determining the quality of the fruit, stabilisation of juices and their de-acidification, demineralisation, clarification and storage, preparation of concentrates and reconstitution of juices from them.

Work on black currant juice has shown that the content of ascorbic acid is reduced by nitrogenous manuring but that this treatment, by increasing fruit yields, also increases the amount of ascorbic acid produced per acre.

The losses of ascorbic acid in jams and in bottled products stored in various ways and using different methods of sealing have been examined.

Assays have been carried out of vitamins of the B group—nicotinic acid, biotin and pantothenic acid—in several fruits and vegetables. Broad beans, among the vegetables, gave outstandingly high values.

**Other Activities.**

*Members' Day.* The annual field day for members was held on Tuesday, July 13th, in combination with the Annual Meetings of the Governors and Members of the National Fruit and Cider Institute. A very varied programme had been prepared, including the following items:

1. Field plots—including bush cider orchard, method of cover crop management, black currant culture, effects of mineral deficiencies in fruit and vegetable crops.

2. Pot culture exhibits—demonstration of injurious factors in acid soils, problems of iron deficiency in crops.

3. Workshops and machinery—types of high pressure spray pumps, use of colloid mill to prepare spray emulsions, demonstration of an automatic sprayer and power duster.

4. Laboratory work—rapid chemical tests for nutrient status of crops, use of radio-active tracers in plant nutrition studies, action of growth regulating substances, methods for home bottling and canning, effects of new systemic insecticides.

5. Cider house—farm orchard surveys, plant and equipment used in cider-making.

*Cider Sampling Day.* The annual sampling day was held on May 6th. It followed the same general lines as those of the previous post-war years. This time particular attention was given to single-variety ciders made from the varieties recommended for future propagation by the Cider Advisory

Committee. Another group of ciders illustrated the effect of specific gravity upon flavour balance in relation to the respective acid and tannin contents. Other ciders sampled were made from individual little-known varieties which had been sent to the Station for trial of vintage quality.

Since the 1947 perry pear juices were considerably above the average in vintage quality a group of single-variety perries was also included in the sampling programme.

A new feature was a collection of compounded drinks made with cider as the base and flavoured with various fruit juices and spirits. The purpose was to illustrate the use of cider for the making of cocktails and fruit cups.

Exhibits relating to current activities of the Cider Section were also staged.

During the afternoon the trial cider bush plots and orchards were visited and demonstrations of spraying and orchard management given.

*Visitors.* Fifty-five organised parties visited the Station during the year and applications from several others who wished to come had to be refused owing to lack of guides. It should be pointed out that the Station has only one part-time official guide to receive parties and that reception on the present scale is only possible by the help of members of the research staff for the more specialised parties, which unfortunately means interference with work and often considerable sacrifice of personal leisure. The parties included three from abroad—Danish fruit growers, La Ligue Pomologique Belge and Swedish students attending a Summer School at the University. Other parties requiring mention were the Vice-Chancellors of Empire Conference, Society of Experimental Biology, Mid-Southern Section of the Royal Institute of Chemistry, National Agricultural Advisory Service Horticultural Officers for the S.W. Province, Western Province Officers and Provincial Grassland and Crops Committee, Informal Conference of the Agricultural Research Council Mineral Deficiency workers, and Ministry of Agriculture National Agricultural Advisory Service Fruit Group Specialist Officers. The full list is as follows :

Agricultural Research Council Mineral Deficiency Workers.
Berkshire Fruit Growers.
Bethel Youth Fellowship, Bristol.
Birmingham University Biochemical Society.
Bristol Dental Students Association.
Bristol Gardeners' Association.
Bristol University Chemical Society.
Bristol University Domestic Science Students.
Bristol University Employees' Association.
Bristol University Mathematical Society.
Bristol University Microbiological Students. (2).
Burderop Park Training College.
Clifton College Young Farmers' Club.
Colston's Girls' School.
Danish Fruit Growers.
Diocesan Training College.
Eden Grove Methodist Churchmen's Discussion Group.
Federation of Bristol Old Boys' Societies.
Henbury and District Young Farmers' Club.
Hereford Training College.
Horfield Townswomen's Guild.
Institute of Handicraft Teachers.
Kingswood Secondary Boys' School Gardening Class.
" La Lique Pomologique Belge."
Long Ashton Horticultural Society.
Long Ashton Womens' Institute.
Mardon's Evening Club, Bristol.
Martock Gardening Society, Somerset.

Martock Young Farmers' Club, Somerset.
Ministry of Agriculture and Fisheries Fruit Group.
Monmouthshire Agriculture Institute Students.
Nottingham University School of Agriculture Horticultural Students.
National Agricultural Advisory Service, S.W. Province Horticultural Officers.
National Agricultural Advisory Service, Provincial Grass and Crops Committee.
National Agricultural Advisory Service, S.W. Province Headquarters Officers.
Oakley Training College for Men.
Paignton and District Horticultural Society.
Plas Dinam Horticultural Students.
Reading University Students.
Redland Training College Gardeners' Club.
Royal Institute of Chemistry, Mid-Southern Counties Section.
Society of Experimental Biology.
Somerset Farm Institute, Cannington, Students. (2).
South Wales and West Midland Branch of Horticultural Education Association.
South West Branch of Horticultural Education Association.
Street and District Allotment Society.
Studley Horticultural Students.
Swedish Students of Bristol University Summer School.
Vice-Chancellors of Empire.
Waterperry Horticultural School.
Whitchurch Evening Institute Horticulture Class.
Whitelands College, Putney, Students.
Worcester Training College Students.

A large number of visiting scientists from overseas countries were again welcomed at the Station, among whom we may mention specially Dr. R. N. Robertson, Principal Plant Physiologist, Division of Food Preservation, Sydney, Mr. D. Martin, senior Research Officer, Australian Scientific Research Liaison Officer, Mr. R. L. Crocker, Waite Institute, S. Australia, Mr. G. Stephens, Division of Soils, C.S.I.R., Mr. F. M. Read, Chief Inspector of Horticulture, Melbourne, all from Australia ; Dr. E. G. Ballard, Plant Diseases Division, C.S.I.R. and Dr. Grange, Soil Survey, from New Zealand ; Dr. C. E. Kellogg, Chief of Soil Survey Division, Bureau of Plant Industry, Mr. R. E. Pendleton, Johns Hopkins University and Professor Charles J. Willard, Department of Agronomy, Ohio State University, from U.S.A. : Dr. Banga, Director of the Institute of Horticultural Botany, Wageningen, Dr. G. de Bakker, Inspector of Horticultural Research, Prof. S. T. Wellensiek, Horticultural Commissioner, Wageningen, and Dr. Temme, Chief Agricultural Adviser, Potash Limited, from Holland. Dr. Nils Dullum, State Experimental Station, Blangstedgaard, Mr. I. Petersen, Research Station, Lyngby, from Denmark ; and Dr. O. Arrhenius, Grödinge, Miss Berta Danielsson, Cytologist, Alnarp, and Mr. Hakanson, Secretary, Swedish Agricultural Bureau of Information, from Sweden.

Members of staff were glad of the opportunity to meet these overseas colleagues and to discuss with them problems of mutual interest.

*Shows and Demonstrations.* In accordance with the new arrangement whereby the main responsibility for show exhibits rests with the National Agricultural Advisory Service, these were strictly limited, and were staged to meet special needs. The following is the list of shows and exhibitions to which the Institute contributed :

*Royal Horticultural Society*—Chelsea Show, May 26th to 28th, 1948.
*Bath and West Show*—Cardiff, May 26th to 28th, 1948.
*Three Counties Show*—Gloucester, June 8th to 10th, 1948.
*Bristol Horticultural Show*—Bristol, July 31st, 1948.
*Royal Horticultural Society*—London, September 7th, 1948.
*Bath Horticultural Show*—Bath, September 15th, 1948.

*Students and Voluntary Workers.* The number of research students has remained severely limited due to the lack of bench space. Mr. R. J. W.

Byrde, a student registered for the degree of Ph.D. at Reading University, is spending a proportion of his time at Long Ashton to work under Mr. Marsh by special arrangement with Professor R. H. Stoughton and Reading University. Mr. Byrde is making a special study of Brown Rot of Apple and Plum.

In the Cider Section, Mr. K. S. Showering, Shepton Mallet, Mr. C. Hartley, Fairoak, and Mr. T. G. Davies, Taunton, have taken practical courses in Cider making.

In the Domestic Preservation Section the following courses were given : a one-day refresher course in January for senior demonstrators of Food Advice Centres at London ; in April, a two-day course for junior demonstrators at Eastbourne ; in May and June, three three-day courses for training W.V.S. organisers in the running of a Fruit Canning Centre ; the usual summer courses in July, August and September, at Long Ashton, in domestic fruit and vegetable preservation and judging. Thanks are due to Miss P. Leicester for assistance given in connection with these courses.

Mr. J. E. Beviss has again helped in the Plantations and with experimental plots, for which sincere thanks are due.

*Acknowledgments.* Members of the senior staff have again undertaken special work in addition to their normal duties. Dr. Martin has continued to help with the research programme and in advising on special requirements of equipment and apparatus. Dr. Kearns has again given invaluable assistance in connexion with building developments, and in organising and supervising repair work to equipment, machinery and tools both for the laboratories and the farm. Miss Sturdy has been responsible for the running of the canteen and Mr. Marsh has dealt with editorial matters, visitors and parties. Mr. Spinks continued to act as librarian. To these and to other members who have given special help, best thanks are accorded.

Grateful acknowledgment is also made to all members of the assistant and technical staffs and to all employees for their loyal support in carrying through the research programme during the year.

# A NOTE ON THE UNFRUITFULNESS OF A ROGUE STRAIN OF THE BLACKCURRANT VARIETY "INVINCIBLE GIANT PROLIFIC."

By L. C. LUCKWILL.

## Introduction.

Potter in 1948 (1) first drew attention to the fact that there were two distinct forms of the blackcurrant variety "Invincible Giant Prolific" in distribution. The true variety, which is said to be indistinguishable from Goliath, is a good cropper; the rogue strain, on the other hand, although a vigorous grower, sets only a very sparse crop, and, when planted in large blocks, often fails entirely to fruit.

Apart from its fruiting behaviour the rogue strain differs from the true variety in many vegetative characters, the chief of which are summarised in the following table, based on unpublished descriptions by Hughes and Todd (4):—

| Character | Invincible Giant Prolific (and Goliath) | Invincible Giant Prolific (Rogue) |
|---|---|---|
| Central lobe of mature leaf | Broad, regular, tri-lobed | Broad, tapering, long. |
| Basal sinus | Medium, broad. | Deep and wide. |
| Leaf surface | Little rugose, margins much recurved and central lobe curved under. | Flat. |
| Petioles | Medium, green at base. | Very short, pink blotch at base. |
| Winter buds | Longish, oval-conical.<br>Scale 'tips' long.<br>White-green, tinged little pink. | Plump, cob-nut shaped.<br>Scale 'tips' short.<br>Salmon brown. |
| Rachis | Very dark carmine. | Green slightly pink. |
| Pedicel bracts | Very long, linear. | Medium. |

Recent investigations have revealed that this unfruitful rogue strain is now widely distributed throughout the country, and that the vast majority of the bushes in cultivation under the name Invincible Giant Prolific are in fact of this type.

During the winter of 1947-48 a four-year old bush of this rogue, which had failed to fruit the previous season, was transplanted into a glasshouse at Long Ashton, and preliminary observations on the cause of unfruitfulness and possible methods of overcoming it were made. In the Spring of 1948, with the co-operation of Mr. Fry, Horticultural Officer for Lindsey, observations were made on a large block of unfruitful bushes in Lincolnshire, and spraying trials with various growth-regulating substances were carried out. The results of these experiments and observations are reported in this note.

**Pollination Experiments.**

The bush in the glasshouse flowered profusely in the early Spring of 1948 and the flowers appeared normal in structure. Although the style was rather long and the stigmas of most flowers protruded beyond the level of the stamens, this character was no more marked than in some other varieties, such as Boskoop Giant, which crop well in the field. Moreover, the anthers produced abundant pollen which germinated well in culture, so it is clear that the unfruitfulness cannot be attributed to male-sterility.

All the flowers on several branches of the bush were self-pollinated by hand as they opened, other branches being left untreated. No fruit set on any of these branches and the flowers shrivelled and abscissed from the rachis within one to two weeks. Flowers on other branches of the same bush were hand-pollinated with pollen collected from Baldwin and Mendip Cross. In marked contrast to the selfed flowers, all the cross-pollinated flowers set normally, and these branches carried a full crop of fruit which developed to maturity.

Microscopic sections were prepared of " selfed " and " crossed " flowers at various times after pollination. Examination of these showed that there was little difference in the rate at which the pollen tubes grew down the style, and in both cases abundant pollen tubes could be seen in the ovarian cavity three days after pollination. In the " selfed " flowers, however, although many of the pollen tubes lay in close proximity to an ovule, no fertilization or embryo development was observed, and all the ovules collapsed and shrivelled within a few days.

The unfruitfulness of this strain of blackcurrant therefore appears to be due to self-incompatibility of an unusual type. In most previously investigated cases of incompatibility (e.g. Plums and Sweet Cherries) the failure to set fruit is attributable to the slow growth of the pollen tubes in the stylar tissue, the tubes thus failing to reach the ovary before the style abscisses or the ovules abort. In this instance, however, the pollen tubes quickly reach the ovules, and incompatibility is due to the failure of fertilization.

It is of interest to note that a previous case of self-incompatibility in blackcurrants was reported from Belgium in an unidentified variety grown under the name of Lee's Prolific (2, 3). In this case the pollen tubes were observed to enter the micropyle, but fusion of the male and female gametes failed to occur. In " Invincible Giant Prolific " it was not possible to say from the material available whether the pollen tubes actually entered the ovary, but the incompatibility seems to be of an essentially similar type to that reported for " Lee's Prolific."

The observation that this strain of blackcurrant will set fruit only when cross-pollinated is supported by observations of its behaviour in the field. In a block of 2,400 bushes of this variety in Lincolnshire the only bushes to carry any fruit in 1948 were those adjoining a block of Wellington XXX, and these set a very meagre crop. This observation suggests that, when planted close to other varieties there is sufficient natural cross-pollination to enable this strain to set a partial crop, but that when planted in large blocks, with no opportunity for cross-pollination, it fails entirely to fruit.

**Origin of the Rogue Strain.**

The variety Invincible Giant Prolific was raised in 1915 by the late Mr. D. Storrie, but there appears to be no definite information as to how or when the rogue strain first made its appearance.

It may possibly be a bud sport of Invincible Giant Prolific or Goliath, which, on account of its greater vegetative vigour was propagated in preference to the true variety. If this were so one would expect that occasionally the unfruitful form would throw a reverse sport to the original variety. Inspection of the Lincolnshire plantation already referred to did, in fact, reveal three bushes which were fruiting normally, all of which appeared to be of the true Goliath type. On the other hand, none of the rogue bushes were observed to carry individual fruiting branches of Goliath, as might have been expected on this hypothesis. Moreover the rogue is so entirely distinct in its vegetative characters as to suggest that it may have arisen as a chance seedling, having no relation to the true variety.

Whatever the explanation of its origin it appears that the two forms became mixed and that the unfruitful rogue was distributed under a false name.

**Trials with growth-regulating substances.**

In some plants it is possible to stimulate fruit development without pollination by the application of certain growth-regulating substances, and it was thought that such treatment might possibly prove effective in setting fruit on this strain of blackcurrant. A spray trial was therefore laid down in the Lincolnshire plantation, and a similar, though smaller scale, trial was carried out using individual branches of the bush in the greenhouse. The following spray treatments were applied at full bloom :—

A. 2-naphthoxy-acetic acid at concentrations of 10, 20, 50 and 100 p.p.m.
B. 2:4 dichlorophenoxyacetic acid (as diethylamine salt) at 1, 5 and 20 p.p.m.
C. 2:4 dichlorophenoxypropionic acid (as diethylamine salt) at 1, 5 and 20 p.p.m.

In the field trial each of the 10 treatments were applied to 10 bushes, control bushes being sprayed with water only. Most of these sprays delayed the abscission of the flowers but none of them stimulated the parthenocarpic development of the fruit. 2:4 dichlorophenoxyacetic acid at 20 p.p.m. caused a rather severe curling of the foliage.

**Recommendations.**

The only possible advice to growers who are unfortunate enough to have plantations of this particular strain of Invincible Giant Prolific, is to destroy the bushes, as there appears to be no practicable way of inducing them to set a good crop of fruit in the field. Close interplanting with a pollinator variety would probably give some fruit, but it is doubtful whether the amount of natural cross-pollination would ever be sufficient to ensure a full crop.

The occurrence and widespread distribution of this unfruitful strain emphasises the need for constant vigilance on the part of the propagator. In this particular case the unfruitfulness was accompanied by a change in vegetative characters which should have enabled the rogue to be detected as soon as it appeared. This may not always be so, however, which suggests that, in selecting material for propagation, particular attention should be paid to the fruiting capabilities of the parent bushes, as well as to their trueness to type and freedom from disease. Only thus can such mistakes be avoided in future.

## ACKNOWLEDGMENTS.

I wish to thank Mr. W. G. Fry, Horticultural Officer for Lindsey, who carried out all the field work in this investigation, and Mr. J. F. D. Hargreaves, on whose plantations the spraying trials were carried out, for their interest and co-operation in this investigation. I would also thank Mr. J. C. Todd for permission to quote from his unpublished interim report on blackcurrant varieties and to Mr. J. M. S. Potter for his criticism of the manuscript.

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# THE EFFECT OF GROWTH SUBSTANCES APPLIED AT FULL BLOOM ON FRUIT SET AND FRUIT DROP IN THE APPLE.

By L. C. LUCKWILL.

## INTRODUCTION.

Thinning of apple and pear crops may be performed for one of two reasons : (a) to increase fruit size and quality in heavy cropping varieties, or (b) to encourage regular cropping in those varieties which otherwise would tend to bear biennially.

Where the object is to improve fruit size thinning is usually deferred until after the " June " drop : biennial varieties in their " on " year, however, must be thinned much earlier than this if increased fruit bud formation for the following year is to be achieved. In the West of England fruit buds can first be distinguished (microscopically) towards the end of June, but the processes leading up to their formation are certainly initiated some weeks earlier than this, probably not long after fruit-setting. Thinning during the blossoming period is therefore highly desirable in such cases, but is rarely practicable where large numbers of trees are involved. Blossom-thinning by hand is a laborious and costly operation and most of the blossom-thinning sprays which have been recommended (such as sodium D.N.O.C., creosote oil and tar oil distillates) are liable to cause severe damage to the foliage and may even kill many of the less vigorous spurs (2). A still more serious deterrent to such early thinning is the uncertainty as to what proportion of the remaining fruitlets will be shed in the " first " and " June " drops. What is required therefore, is a method of reducing fruit set which will not damage the foliage and which will, at the same time, prevent or greatly reduce the early waves of fruitlet abscission. It was thought that sprays of certain growth substances, applied at full bloom, might adequately perform both these functions.

Alpha-naphthalene-acetic acid and related compounds at strengths of 10 to 20 p.p.m. have been employed with some success in America as blossom-thinning agents on apples (1) and similar sprays are known to be effective

in reducing the preharvest fruit-drop of many varieties. More recently Vyvyan, at East Malling, has shown that growth substances can be used to control June drop as well, at least in the variety Cox's Orange Pippin (3).

During 1948, therefore, a small scale trial was laid down at Long Ashton to investigate the possibility of using growth substances, applied at full bloom, for the double purpose of reducing fruit set and controlling the early periods of fruit drop.

**Experimental Methods.**

The experimental material consisted of a row of uniform trees of the apple variety Lane's Prince Albert growing under arable conditions. The trees were 25 years old and carried a heavy crop of blossom buds. They received the following spray treatments :—

*Tree* 1. 2 : 4 dichlorophenoxy-acetic acid (2 : 4 D) at a concentration of 1 part per million in water, at full bloom.
*Tree* 2. Alpha-naphthalene-acetic acid (ANA) at 5 p.p.m., at full bloom.
*Tree* 3. Control—no spray.
*Tree* 4. No spray at full bloom but ANA at 10 p.p.m. was applied 30 days after petal fall, just prior to the " June " drop.
*Tree* 5. Control—no spray.
*Tree* 6. 2 : 4 D at 1 p.p.m. + ANA at 5 p.p.m. at full bloom.
*Tree* 7. Control—no spray.
*Tree* 8. 2 : 4 D at 1 p.p.m. + Indolyl-acetic acid (IA) at 5 p.p.m. at full bloom.
*Tree* 9. ANA at 5 p.p.m. + IA at 5 p.p.m. at full bloom.
*Tree* 10. 2 : 4 D at 1 p.p.m. + IA at 5 p.p.m. + ANA at 5 p.p.m. at full bloom.
*Tree* 11. IA at 5 p.p.m. at full bloom.

The various treatments were distributed at random amongst the experimental trees, but sufficient trees were not available to replicate all the treatments. The results could not therefore be analysed statistically and the present experiment must consequently be regarded as preliminary in nature only.

0·25% ester salts was added to all the sprays as a wetting agent. The sprays were applied with power spraying equipment using an average of about 8 gallons of wash per tree. This was sufficient to give a good drenching spray. With the exception of tree 4, which was sprayed 30 days after petal fall in an attempt to control the " June " drop, the trees were sprayed on May 6th, under almost ideal conditions. The day was warm and sunny and there was very little breeze, so that spray drift was reduced to a minimum. On this day the trees were just past full bloom and the first petals were beginning to fall. Tree 4 was sprayed on June 9th under dull but calm conditions. One hour after spraying light rain began to fall and it was therefore considered advisable to repeat this spraying two days later under more favourable conditions.

The following observations were made on the experimental trees :—

*Percentage set.* By percentage set is meant the percentage of blossoms in which the ovary started to swell into a fruitlet, irrespective of whether that fruitlet later abscissed or not. This was estimated as follows : two large branches on each tree were chosen and the total number of blossoms on each

branch counted. Seven days after petal fall, before the "first" drop had started, a count was made of the number of developing fruitlets on each branch and from this the percentage set was calculated.

*Fruit drop.* Fruit drop was measured by collecting all the dropped fruitlets from under each tree at weekly intervals from the date of petal-fall (May 10th). In order to do this it was necessary to carefully weed and level the ground beneath each tree, and then to roll it smooth with a heavy roller. This operation had to be repeated several times during the season in order to keep the ground weed free. The drops were classified as either physiological, (sound fruitlets), or pathological, (fruitlets attacked by Sawfly, Codlin Moth or Brown Rot), and the number of fruitlets in these different categories were recorded separately.

The mean rate of fruit drop during each week was calculated using the formula recommended by Vyvyan (1946) viz. :—

$$Q = 100 \ (\log_e X_1 - \log_e X_2)/(t_2 - t_1)$$

where Q is the required index of rate of drop during the period $t_1$ to $t_2$, $X_1$ is the number of fruits on the tree at $t_1$ and $X_2$ the number at $t_2$, and $(t_2 - t_1)$ the length of the period in days.

*Fruit picked.* The crop was harvested on September 23rd, records being taken of both the number and weight of fruits picked from each tree. A count was also made of the number of terminal ("king") and lateral fruits in each sample.

*Total number of flowers.* This was estimated from (a) the percentage set, determined as above, and (b) the total number of fruitlets set. The latter was obtained by totalling the number of fruits picked and the number dropped from each tree.

## RESULTS.

*Effect of sprays on foliage.* The day after spraying all the treated trees showed a slight bending or twisting of the leaf petioles, but apart from this no other effects of the sprays on the foliage were observed. Neither was the subsequent vegetable growth of the sprayed trees in any way impaired.

*Effect of sprays on fruit set.* It is clear from Table I that all the sprays applied at full bloom were very effective in reducing the set of fruits. The mean set for the controls (including tree 4, which was not sprayed until later) was 21·4%. Since there was an average of 5·7 blossoms per cluster, this means that the natural set averaged just over one fruitlet per cluster. The mean set for all the sprayed trees, on the other hand was reduced to 5·5%, or about one fruitlet per three clusters.

The most effective spray seems to have been that combining all three growth substances (tree 10), though in the absence of replications little weight can be attached to the comparatively small differences in set between the different treatments.

*Effect of sprays on fruit drop.* (*a*) *physiological drop.* The data on fruit drop is summarised in Table II, in which the number of fruits dropped from each tree is recorded as a percentage of the number of fruitlets set. It will be seen that the growth substance sprays had very little effect on the total physiological drop; if anything there was a tendency for the sprayed trees to show an even greater drop than the unsprayed controls, the mean drop for the latter being 32·8% compared with 41·4% for the treated trees. The variation in drop between different trees in the row appeared to be correlated with the general vigour of the tree rather than with any of the treatments

TABLE I.

EFFECT OF GROWTH SUBSTANCES ON FRUIT-SET AND CROPPING OF LANE'S PRINCE ALBERT. NO. OF FRUITS EXPRESSED AS % OF NO. FLOWERS ON TREE.

| Tree No. | Spray mixture (concs. in p.p.m.) | Stage when applied | No. of flowers on tree | Fruitlets set | | Fruit picked | |
|---|---|---|---|---|---|---|---|
| | | | | No. | % | No. | % |
| 7 | Control | — | 13,260 | 2982 | 22·5 | 277 | 2·09 |
| 3 | Control | — | 14,653 | 3473 | 23·7 | 223 | 1·54 |
| 5 | Control | — | 14,987 | 3120 | 20·8 | 185 | 1·23 |
| 1 | 2 : 4 D (1) | Full bloom | 10,410 | 699 | 6·7 | 146 | 1·40 |
| 2 | ANA (5) | ,, | 9,680 | 494 | 5·1 | 113 | 1·17 |
| 11 | IA (5) | ,, | 5,500 | 176 | 3·2 | 8 | 0·14 |
| 6 | 2 : 4 D (1) + ANA (5) | ,, | 33,300 | 1265 | 3·8 | 153 | 0·46 |
| 9 | ANA (5) + IA (5) | ,, | 7,300 | 709 | 9·7 | 46 | 0·63 |
| 8 | 2 : 4 D (1) + IA (5) | ,, | 17,130 | 1270 | 7·4 | 224 | 1·31 |
| 10 | 2 : 4 D (1) + ANA (5) + IA (5) | ,, | 20,100 | 564 | 2·8 | 63 | 0·31 |
| 4 | ANA (10) | Petal-fall + 30 days | 12,393 | 2297 | 18·5 | 223 | 1·80 |

TABLE II.

EFFECT OF GROWTH SUBSTANCES ON ABSCISSION OF FRUITLETS IN LANE'S PRINCE ALBERT. DROPS EXPRESSED AS % OF *fruitlets set*.

| Tree No. | Spray mixture (concs. in p.p.m.) | Stage when applied | No. of fruitlets set | Physiological drops | | Pathological drops | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Sawfly | | Codlin | | Brown rot | |
| | | | | No. | % | No. | % | No. | % | No. | % |
| 7 | Control | — | 2982 | 772 | 25·8 | 1899 | 63·5 | 5 | 0·17 | 29 | 0·97 |
| 3 | Control | — | 3473 | 1253 | 36·2 | 1976 | 57·0 | 15 | 0·43 | 6 | 0·17 |
| 5 | Control | — | 3120 | 1139 | 36·5 | 1778 | 57·0 | 5 | 0·16 | 13 | 0·41 |
| 1 | 2 : 4 D (1) | full bloom | 699 | 279 | 39·9 | 208 | 29·8 | 8 | 1·14 | 58 | 8·30 |
| 2 | ANA (5) | ,, | 494 | 183 | 37·1 | 140 | 28·4 | 3 | 0·61 | 55 | 11·10 |
| 11 | IA (5) | ,, | 176 | 107 | 60·8 | 53 | 30·1 | 0 | 0 | 8 | 4·54 |
| 6 | 2 : 4 D (1) + ANA (5) | ,, | 1265 | 547 | 43·2 | 505 | 39·9 | 1 | 0·08 | 59 | 4·66 |
| 9 | ANA (5) + IA (5) | ,, | 709 | 378 | 53·3 | 247 | 34·9 | 5 | 0·71 | 33 | 4·66 |
| 8 | 2 : 4 D (1) + IA (5) | ,, | 1270 | 425 | 33·4 | 597 | 47·1 | 4 | 0·31 | 20 | 1·57 |
| 10 | 2 : 4 D (1) + ANA (5) + IA (5) | ,, | 564 | 252 | 44·7 | 198 | 35·2 | 3 | 0·53 | 46 | 8·16 |
| 4 | ANA (10) + 30 days | Petal-fall | 2297 | 898 | 39·0 | 1120 | 53·2 | 30 | 1·30 | 25 | 1·09 |

applied. In particular trees 9 and 11, which showed the highest physiological drops, were observed to be the weakest trees in the row, a difference which is also reflected in the lower number of blossoms produced on these trees (Table I).

Although the sprays had no marked effect on the total physiological

drop they all had a profound effect on the distribution of the drop throughout the season. This point is illustrated in *Fig.* 1 by means of a series of fruit-drop histograms.

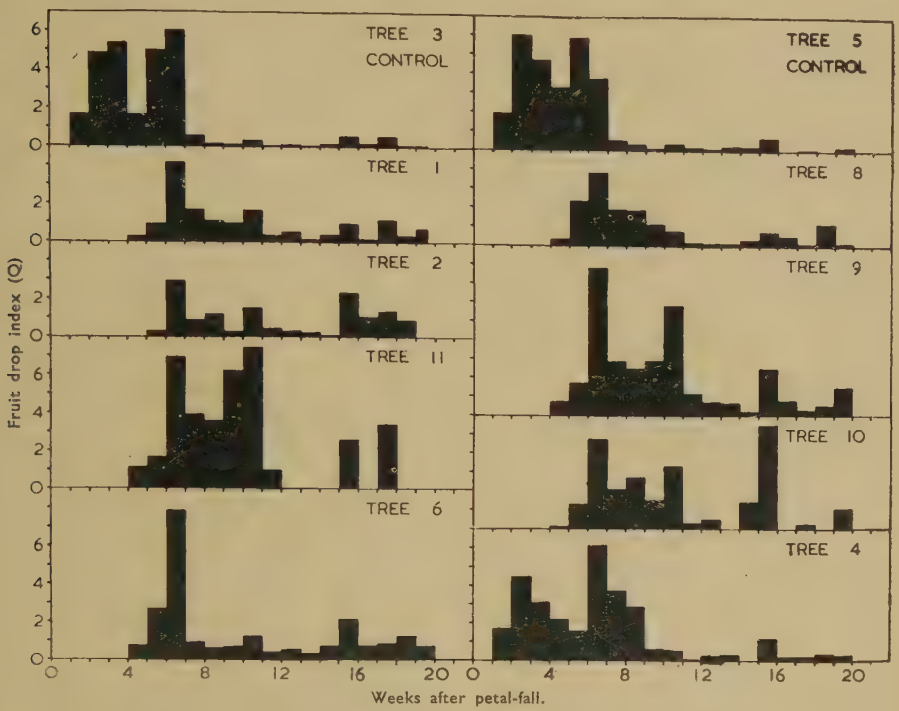


*Fig.* 1. Effect of growth-substance sprays on fruit drop in Lane's Prince Albert. (For treatments see Table II).

In the control trees the physiological dropping of fruitlets (as opposed to unpollinated flowers) started during the second week after petal-fall and occurred in two main waves or peaks. The "first," or post-blossom, drop lasted from the second to the fourth week after petal-fall. There was then a short lull, followed closely by the "June" drop, which lasted from the fifth till the seventh week after petal-fall. In the eighth week there was a very sharp fall in the fruit-drop index, which then remained at a low level until harvest. There was no pre-harvest drop in this variety in 1948.

In none of the trees sprayed at full blossom did the drop begin until the fifth week after petal-fall i.e. a delay of three weeks compared with the controls, and in general the drop was spread over a much longer period than normal. This was especially noticeable in tree 2 which was sprayed with ANA at 5 p.p.m. when in full bloom. In tree 4, however, which was sprayed with ANA at 10 p.p.m. just prior to the "June" drop, the only effect of the spray was to delay the start of the drop by one week and to spread it over a period of three weeks compared with two weeks for the control trees.

*Effect of sprays on fruit drop.* (*b*) *pathological drop.* The usual petal-fall wash of nicotine was not given to the experimental trees as it was not known what effect this might have on the action of the hormone sprays. As a result there was a very heavy infestation of Sawfly which destroyed approximately 60% of the fruitlets set on the control trees. On the trees sprayed with growth substances at full bloom, on the other hand, the Sawfly damage was definitely

less severe, and accounted for an average of only 35% of the set fruitlets. (Table II). There are a number of possible explanations of this observation. In view of the extreme dilutions and non-toxic nature of the substances used it is most unlikely that there could have been any direct toxic action of the growth substances on the Sawfly larvae. On the other hand, the delayed abscission of the fruitlets on the sprayed trees might mean that each larva could spend a longer time feeding in a particular fruitlet, before moving on to attack another one, and consequently might destroy fewer fruitlets during its period on the tree than it would normally do.

There was very little damage by Codlin moth larvae and no outstanding differences between the sprayed and the unsprayed trees were observed.

On the control trees less than 1% of the fruits were infected with Brown Rot (*Sclerotinia fructigena*), the average for the three trees being 0·51%. On the sprayed trees the infestation was much heavier, averaging 6·1% for the seven trees sprayed at full bloom. The attack was particularly heavy on tree 2, on which 11·1% of the fruitlets set were eventually destroyed by this fungus. As in the case of Sawfly damage the effect of the sprays on the spread of Brown Rot is probably an indirect one. On the control trees it was noticed that the infected fruits dropped from the tree quickly and were removed with the rest of the fallen fruits at weekly intervals. On the sprayed trees however, the abscission of the infected fruits, like that of the healthy fruits, was considerably delayed. This meant that such infected fruits hung on the tree, and thus acted as a source of infection for other fruits, over a longer period than they would normally have done, and in this way probably helped the spread of the disease.

*Effect on total crop and proportion of "king" fruits.* The total crop from the control trees averaged 1·62% of the number of blossoms and could be described as light to moderate. The crop on the sprayed trees was definitely light, averaging only 0·77% of the number of blossoms. Expressed as a percentage of the number of blossoms set however, the crop on the sprayed trees was approximately double that on the controls, namely 14·5% as compared with 7·1%, an increase which is entirely attributable to the less severe Sawfly infestation on the trees sprayed with growth substances.

Many blossom-thinning sprays of the caustic type have the disadvantage that they increase the proportion of "king" fruits, which, on account of their larger size, irregular shape and poorer keeping quality, are less desirable than the smaller but more uniform lateral fruits. The comparative immunity of the "king" fruits, developed from the terminal flowers, is due to the fact that these flowers, being the first to open, have often "set" before the spray is applied.

Since nothing is at present known about the mode of action of growth substances in reducing fruit set, it was not known if the same disadvantage would apply to sprays of this type. In order to obtain information on this point therefore, a count was made of the number of "king" and lateral fruits picked from each tree. The following results were obtained :—

TABLE III.

Percentage of "King" Fruits in Crop.

| | No. of trees | Range of variation | Mean |
|---|---|---|---|
| Control | 3 | 1·5— 5·5 | 2·6% |
| Sprayed | 7 | 1·6—12·5 | 5·6% |

The proportion of king fruits in the crop of the control trees was exceptionally low and strongly suggests that there had been a selective thinning of " king " fruitlets, as the result of the heavy Sawfly attack. On the sprayed trees the proportion of " kings " was very variable but, in general, tended to be higher than in the controls. This difference also may be the result of the lighter Sawfly attack on the sprayed trees, rather than a direct effect of the sprays.

*Effect of sprays on parthenocarpy.* In order to test whether any of the sprays used were active in inducing parthenocarpic development of the fruit the following supplementary experiment was carried out :—

Two further trees of Lane's Prince Albert were chosen and, before the blossoms opened, all the clusters on the trees were thinned to 4 buds each. Just before opening each bud was cut in half transversely with a pair of scissors. This removed the anthers and the upper half of the style and effectively prevented pollination, thus ensuring that no normal seeded fruits would develop on either of the experimental trees. The sprays were applied to individual branches on these trees with a small hand sprayer, neighbouring branches being protected from spray drift with large sheets of stiff brown paper. All seven sprays were tested on both trees, and there was also an untreated control branch on each tree.

The flower buds on tree 12 were emasculated on 27th April and the sprays applied the following day during dull windy weather. Rain started three hours after spraying and it was therefore thought advisable to repeat the spraying on May 6th when the weather was sunny, warm, and calm.

Tree 13 was emasculated on April 28th and sprayed on April 30th. The weather was calm, cool and dull, and heavy rain fell within $2\frac{1}{2}$ hours of spraying. No repeat spraying of this tree was made however.

One month after spraying a count was made of the number of fruitlets developing on each branch, and later a record was taken of the number of mature fruits picked. After picking each fruit was cut open to confirm that it was seedless and hence parthenocarpic in origin.

Only a very small proportion of emasculated blossoms set, and from a total of 10,620 blossoms treated only 26 fruits (0·24%) developed to maturity. All these fruits were, however, parthenocarpic. There was much variation in the percentage set between the two trees, even on branches receiving the same treatment, but none of the sprayed branches showed a significantly greater set of parthenocarpic fruits than did the unsprayed control branches.

Although none of the sprays used were effective in stimulating parthenocarpic development, it was observed that they were very active in delaying the abscission of the undeveloped flowers. This applied particularly to sprays containing 2 : 4 D at 1 p.p.m. which delayed flower abscission by 4 to 6 weeks. ANA was considerably less effective in delaying abscission and IA the least effective of the three substances used.

The parthenocarpic fruits developed on trees 12 and 13 were of average weight for the variety but were markedly more conical in shape than normal seeded fruits of this variety.

## DISCUSSION.

The present experiment fully confirms the effectiveness of alpha-naphthalene acetic acid (ANA) and other growth substances as blossom-thinning agents in apples. In recent American work ANA has been employed at concentrations ranging from 10—30 p.p.m., whereas in this experiment

a concentration of only 5 p.p.m., applied at full bloom, was effective in reducing fruit set by approximately 75%. Indolyl-acetic acid and 2 : 4 D were also found to be effective agents for reducing fruit set, the latter being active at a concentration of only 1 p.p.m. Various mixtures of these growth substances were also effective but possessed no particular advantages over the single substance sprays. Such sprays have the great advantage over the caustic type of blossom-thinning sprays that they cause no permanent damage to the foliage and do not impair the growth of the tree in any way.

Unfortunately none of the sprays used had the desired effect of reducing the physiological dropping of the developing fruitlets, and, as a result, the sprayed trees carried only a very light crop. The sprays were, however, very active in delaying the onset of fruit drop and it is possible that had the applications been repeated at monthly intervals a large proportion of the set fruitlets could have been kept on the tree until maturity.

Although growth substances show considerable promise as blossom-thinning agents it is clear that the method has not yet reached the stage when it can be recommended for general use by growers. Further work will be necessary to determine the relation between the time of application of the spray and the reduction in set achieved, and the effect of the sprays on the proportion of " king " fruits in the crop. The present experiment gave no conclusive evidence on the latter point as, even on the unsprayed trees, the proportion of " king " fruits was exceptionally low. This was probably the result of a heavy attack of Apple Sawfly.

## SUMMARY.

(1) Sprays of various growth substances and mixtures of growth substances were applied to trees of Lane's Prince Albert shortly after full blossom in order to determine their effect on (a) fruit set, and (b) fruit drop.

(2) ANA at 5 p.p.m., 2 : 4 D at 1 p.p.m. and IA at 5 p.p.m. were all effective in reducing fruit set without causing damage to the foliage. Mixtures of these substances showed no advantage over the single substance sprays.

(3) All the sprays used were very active in delaying the abscission of unpollinated flowers and growing fruitlets, but the reduced drop during the early part of the season was compensated for by a heavier drop later on.

(4) 60% of the set fruitlets on the control trees were destroyed by Apple Sawfly larvae, but only 35% on the sprayed trees.

(5) None of the sprays used were active in stimulating parthenocarpic development when sprayed on to emasculated blossoms.

(6) ANA at 10 p.p.m., applied just prior to the " June " drop, delayed but did not reduce the drop.

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# CHANGES IN THE BOTANICAL COMPOSITION OF ORCHARD COVER CROPS

By L. C. LUCKWILL.

## INTRODUCTION.

The observations recorded in this note were made in connection with an experiment on the effect of different cover crops on soil fertility and tree nutrition, which was started at Long Ashton in 1946. The general lay-out and management of the experiment have already been described (1), and this report will deal only with the changes which have occurred in the botanical composition of the various cover crops between 1946 and 1948.

The experimental plots, each of approximately half an acre, are situated in an orchard of bush cider apples (Plot 20B) with a spacing of 20 × 20 ft., planted in 1935. From the time of planting until 1944 the orchard was intercropped with blackcurrants and "filler" apple trees and from 1944-46 was kept under clean cultivation. On May 7th, 1946, two of the plots ($A_1$ and $A_2$) were sown with S/23 Perennial Rye Grass, two ($B_1$ and $B_2$) with S/100 White Clover, and two ($E_1$ and $E_2$) with a Rye Grass Clover mixture, and it is these six plots which form the object of the present study.

Management of the plots during the past three years has consisted in close cutting of the sward with a gang-mower at intervals of two to three weeks throughout the growing season, except during the months of September and October when cutting would have interfered with the harvesting of the fruit. All cuttings have been left to rot down *in situ* on the plots. Botanical analysis of the herbage on each plot was made at yearly intervals in the month of August using the method described below.

## METHOD OF ANALYSIS

The percentage frequency (valence) of each species in the sward was determined using a quadrat of 5 × 5 sq. cm. which was thrown 100 times on each plot. By walking up the alleyways between the rows of trees and dropping the quadrat after every three paces, a fairly even distribution of samples over the plot was achieved. Each time the quadrat was thrown a close examination was made of all the herbage within the sample area and each species present was recorded. The percentage of quadrats in which a given species occurred is the percentage frequency of that species.

The estimates of percentage frequency in Table I enable the botanical composition of the different plots to be compared and give a useful indication of which species are increasing and which decreasing. They do not however provide any measure of the percentage area covered or of the density of each species. Thus a species which had a frequency of 90 in 1946, and 45 in 1947, could rightly be assumed to have decreased, but it could not be assumed that it occupied half its former area. Such an assumption would, in fact, be justified only in the limiting case when the area of the quadrat was infinitely small.

In particular it must be remembered that for species with high percentage frequencies (i.e. over 90), small changes in frequency represent relatively large changes in density, and that the reverse is true for species of low frequency. Moreover, a percentage frequency of 0 does not necessarily mean that the species is absent from the plot, but simply that it did not happen to occur in any one of the 100 sample quadrats.

These limitations must be borne in mind when interpreting the data in Table I.

TABLE I.

PERCENTAGE FREQUENCY ANALYSIS OF COVER CROPS (PLOT 20B) OVER THE PERIOD 1946-48.
SIZE OF QUADRAT = 5 × 5 SQ. CM.

| Plot and cover crop sown | Year | Rye Grass | White Clover | Annual Meadow Grass | Creeping Bent Grass | Rough-stalked Meadow Grass | Other annuals | Other perennials |
|---|---|---|---|---|---|---|---|---|
| $A_1$ Rye Grass (S/23) .. | 1946 | 100 | 1 | 72 | 12 | 0 | 26 | 5 |
| | 1947 | 100 | 0 | 18 | 32 | 3 | 0 | 0 |
| | 1948 | 98 | 2 | 37 | 46 | 18 | 0 | 3 |
| $A_2$ ,, ,, ,, .. | 1946 | 99 | 1 | 50 | 12 | 0 | 22 | 5 |
| | 1947 | 99 | 0 | 39 | 30 | 2 | 1 | 5 |
| | 1948 | 97 | 1 | 41 | 40 | 16 | 0 | 4 |
| $B_1$ White Clover (S/100) .. | 1946 | 5 | 99 | 98 | 24 | 0 | 93 | 19 |
| | 1947 | 64 | 93 | 39 | 30 | 0 | 3 | 4 |
| | 1948 | 25 | 46 | 0 | 90 | 7 | 0 | 7 |
| $B_2$ ,, ,, ,, .. | 1946 | 9 | 97 | 100 | 5 | 0 | 54 | 11 |
| | 1947 | 49 | 92 | 83 | 32 | 0 | 0 | 4 |
| | 1948 | 25 | 56 | 8 | 70 | 2 | 0 | 10 |
| $E_1$ Rye Grass (S/23) and White Clover (S/100) | 1946 | 99 | 81 | 81 | 12 | 0 | 23 | 7 |
| | 1947 | 98 | 59 | 32 | 21 | 1 | 0 | 5 |
| | 1948 | 91 | 69 | 49 | 36 | 7 | 0 | 2 |
| $E_2$ ,, ,, ,, .. | 1946 | 100 | 82 | 64 | 6 | 0 | 29 | 5 |
| | 1947 | 95 | 35 | 37 | 13 | 0 | 0 | 0 |
| | 1948 | 94 | 62 | 48 | 26 | 7 | 0 | 4 |

## RESULTS.

*Plots $A_1$ and $A_2$.* Sown with Perennial Rye Grass (Strain S/23) at rate of 20 lb./acre (May 1946).

By August 1946, the date of the first sampling, the Rye Grass on both these plots was well established, and it has succeeded in maintaining itself reasonably well through 1947 and 1948. It is now definitely decreasing in density, however, and is being replaced by Creeping Bent (*Agrostis alba* var. *stolonifera*). This grass, at first present in only small amount, has shown a rapid build-up during the past three years, and can be expected to replace Rye Grass as the dominant species within the next few years. It is an indigenous grass particularly well suited to the heavy soil and high rainfall at Long Ashton, and seems to thrive especially well beneath the shade of the trees. In such situations it is more abundant than between the trees, where the sample quadrats were taken. The figures in Table I therefore give an underestimate of the present abundance of this species on the plot as a whole.

Chief competitor of the Rye Grass during the first year was Annual Meadow Grass (*Poa annua*), which was so abundant that it could be described as co-dominant. In 1947, *Poa annua* was far less abundant, but this was possibly due to the unusually dry summer, as it again increased in 1948. This grass, on account of its lowly habit and the great rapidity with which it can seed itself, proved far less susceptible to continual mowing than the other annual weeds, which had been practically eliminated by the second season. Perennial weeds, on the other hand, have tended to persist, but being present in only very small quantity, have not proved troublesome on these plots.

The only other native grass which has shown any tendency to increase is the Rough-stalked Meadow Grass (*Poa trivialis*), but this is still present only in small quantity, and cannot be considered a serious competitor of *Agrostis*.

The Rye Grass on both plots A and E is parasitized by the rust *Puccinia Lolii* Niels. The infestation was particularly severe in the year of sowing and may have helped to reduce the vigour of this species.

*Plots $B_1$ and $B_2$.* Sown with White Clover (Strain S/100) at the rate of 4 lb./acre (May 1946).

The flora of both these plots has undergone major changes during the past three years. By 1947 it was clear that the S/100 strain of White Clover would not stand up to frequent cutting with the gang mower, and was decreasing in density. By the summer of 1948 it had entirely disappeared from beneath the trees, though still present in relatively small quantity between the trees. Even here however it is being rapidly replaced by Creeping Bent which is now the dominant plant in the sward. This change is somewhat less marked on plot $B_2$ than on $B_1$, where the original amount of indigenous *Agrostis* was higher than on any of the other plots.

Although the White Clover germinated well the somewhat upright habit of this particular strain resulted in rather a thin sward and provided ideal conditions for the germination of weed seeds, with the result that the incidence of weed species is far higher on these plots than on any others. In the summer of 1946, 23 different weed species were recorded within the sample quadrats on these plots, compared with only 14 for plot A and 17 for plot E.

Annual weeds, including Chickweed, Shepherd's Purse, Annual Sow Thistle and Field Speedwell were particularly abundant, but had practically disappeared by the second season. Of the perennial weeds which established themselves in the first season, Creeping Buttercup, Broad-leafed Dock, and Dandelion, have tended to persist, and are still relatively abundant on these plots.

As on plot A *Poa annua* was the most abundant weed species in the first year, being present in almost every sample quadrat, but by 1948 it had been almost eliminated. This is in marked contrast to the situation in the Rye Grass plots where *Poa annua*, in spite of its lower initial frequency, is still very abundant. The exceptionally rapid spread of *Agrostis* on the Clover plots may be the explanation of this difference, as the spreading mat-forming habit of this grass appears to be very effective in smothering small annual weeds.

*Plots $E_1$ and $E_2$.* Sown with Perennial Rye Grass (Strain S/23) at rate of 20 lb./acre, and White Clover (Strain S/100) at rate of 2 lb./acre. (May 1946).

These plots might be expected to have been intermediate in their behaviour between those sown with Rye Grass only and those sown with White Clover only, but this expectation has, in fact, been only partially fulfilled.

As on the A plots the Rye Grass is gradually dying out and being replaced by *Agrostis*. The latter however, has not increased as rapidly here as on the other plots, as the percentage frequency estimates show. The somewhat heavier rate of initial seeding on this plot might perhaps be responsible for the slower build-up of *Agrostis*; alternatively, light intensity may be an important factor. It should be noted (1) that these two plots adjoin one another on either side of the central alleyway dividing the two experimental blocks and the edges of these plots therefore are less shaded than those of non-marginal plots. The Rye grass was markedly more vigorous along this central alleyway than in the more shaded area, and the *Agrostis* correspondingly less successful as an invader.

The same factor may be partially responsible for the greater success of White Clover on this plot, where, after a temporary decline during the dry summer of 1947, it was again on the increase in 1948. To some extent it seems that the presence of Rye Grass may " protect " the White Clover, as these two species are able to grow together in intimate association. This is not possible however in a Clover-*Agrostis* sward, where the Clover quickly becomes smothered out by its more vigorous neighbour.

The most abundant weed species on the Rye Grass/Clover plots is still *Poa annua*, which has maintained itself here to about the same extent as on the Rye Grass plots. Other annual and perennial weeds have also shown a similar behaviour on these two plots.

## SUMMARY AND CONCLUSIONS.

It is clear from the above observations that none of the three cover crops studied can be regarded as a stable plant community, and that all have undergone very considerable changes in composition during the first three years after sowing. Moreover, it appears that these changes are still in progress and that on all the plots, irrespective of the original composition of the sward, they show a similar trend ; namely, towards the replacement of the sown species by Creeping Bent Grass ( *Agrostis alba* var. *stolonifera*). The composition of the original seed mixture appears to be of importance only in so far as it alters the rate at which this change occurs. Where the S/100 strain of White Clover alone is sown, its replacement by *Agrostis* is rapid, and is almost complete within three years. This particular strain of Clover is intolerant of gang mowing and does not seem to thrive well beneath the shade of trees.

Rye Grass proved a more successful opponent of Agrostis, but that too is now disappearing rather rapidly and will probably not persist for more than a few years longer.

The Rye Grass/Clover mixture proved the most stable of the three crops studied, and it is on these plots that the *Agrostis* has made least progress. This may be due to the rather higher light intensity on these plots, resulting from their marginal position, though it appears that, to some extent, these two species may be able to " protect " one another through their ability to grow in intimate association.

These facts suggest that where it is desired to establish a permanent and stable cover crop, attention should be directed to the possibility of using *Agrostis alba* var. *stolonifera* itself. Although usually regarded as an inferior grass in agriculture it would appear to have many advantages as a cover crop plant for orchards in districts where the summer rainfall is relatively high. These may be briefly summarised as follows :—

(1) It is very hardy and persists well on damp soils.
(2) It grows well beneath the shade of trees.
(3) It forms a close sward, very resistant to treading.
(4) It is resistant to close cutting.
(5) Its vigorous mat-like growth is very effective in smothering annual weeds.
(6) Its chief period of growth is in late summer and autumn, so that it would not be in active competition with the trees during their growing period in Spring and early summer.

Against these advantages must be set the fact that it is a shallow rooted species which would not stand up well to periods of summer drought. Moreover, good samples of seed are difficult to obtain, though where conditions are suited to its growth a very light seeding would probably suffice for rapid establishment.

REFERENCES.

(1) Bould, C. (1946). Cover crops in relation to soil fertility and tree nutrition. An experiment with bush cider trees. Progress report 1. *Ann. Rpt. Long Ashton Res. Stn.*, 1946; 23–31.

# COVER CROPS IN RELATION TO SOIL FERTILITY AND TREE NUTRITION. AN EXPERIMENT WITH BUSH CIDER TREES.

## PROGRESS REPORT—II.

By C. BOULD, J. A. H. TOLHURST and R. M. JARRETT.

A description of the layout, management and aims of this experiment was given in progress report I (1). The aims are twofold; firstly to study the effect of certain cover crops and clean cultivation on soil fertility and the utilization of fertilisers, and secondly to determine the effect of treatments on the growth, crop yield, and leaf nutrient status of apple trees.

The treatments on one half of the plot (20 B) are :—

A. Perennial rye grass S.23.
B. White clover S.100.
C. Annual cover crops. Vetch followed by winter rye.
D. Clean cultivation.

All sub-plots on 20 B receive an annual dressing of N.P.K. in the spring at the following rates :—

N = 3 cwts. nitro-chalk
P = 3 cwts. superphosphate } per acre
K = 1 cwt. muriate of potash

The cover crops are kept short by frequent gang-mowing, and the annual cover crops are ploughed in whilst green. The botanical composition of the perennial covers has changed considerably since seeding (2). Perennial rye grass has been replaced to a large extent by *Agrostis alba* var. *stolonifera*. This grass has also steadily increased on the clover plots. White clover (S.100) has not persisted under the type of management practised in this experiment. It has been decided, therefore, to plough up the clover plots and re-seed with Cotswold Wild White Clover, a more prostrate and persistent type.

This report deals with the chemistry of the soil and leaves, together with information on crop yield and girth increase.

## EXPERIMENTAL.

### Soil Analysis.

Soil samples were taken on a sub-plot basis from plot 20 B on 1.3.46 at depths of 0—6″, 6—12″ and 12″+. The analytical results from these samples are given in Table I.

TABLE I.

ANALYSIS OF SOILS FROM PLOT 20 B. PERCENTAGES EXPRESSED ON AN AIR DRY BASIS. SAMPLED 1-3-46.

| Sample and Depth | Plot | Organic C | Total N | Easily reducible Mn. p.p.m. | $CaCO_3$ | pH | 1% Citric sol. $P_2O_5$ | Exchangeable | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Mg | K |
| S1 0—6″ | C1 | 2·60 | 0·28 | 118 | 2·3 | 7·7 | ·018 | ·006 | ·024 |
| 6—12″ | | 1·64 | 0·17 | 143 | 1·0 | 7·7 | ·008 | ·005 | ·013 |
| 12″+ | | 0·55 | 0·06 | 298 | Trace | 7·5 | ·005 | ·004 | ·010 |
| S2 0—6″ | D1 | 2·89 | 0·30 | 87 | 3·1 | 7·8 | ·015 | ·007 | ·020 |
| 6—12″ | | 1·89 | 0·19 | 93 | 1·7 | 7·9 | ·007 | ·005 | ·007 |
| 12″+ | | 0·66 | 0·07 | 234 | Trace | 7·8 | ·006 | ·003 | ·008 |
| S3 0—6″ | B1 | 3·00 | 0·28 | 118 | 1·4 | 7·8 | ·022 | ·006 | ·018 |
| 6—12″ | | 1·48 | 0·15 | 270 | 0·8 | 7·7 | ·010 | ·005 | ·009 |
| 12″+ | | 0·51 | 0·05 | 348 | Nil | 7·6 | ·008 | ·003 | ·007 |
| S4 0—6″ | A1 | 3·38 | 0·35 | 111 | Nil | 5·7 | ·023 | ·008 | ·026 |
| 6—12″ | | 1·62 | 0·17 | 270 | Nil | 6·2 | ·005 | ·007 | ·011 |
| 12″+ | | 0·55 | 0·06 | 360 | Nil | 6·4 | ·003 | ·007 | ·009 |
| S5 0—6″ | E1 | 3·30 | 0·34 | 113 | Nil | 6·0 | ·028 | ·009 | ·028 |
| 6—12″ | | 1·58 | 0·16 | 268 | Nil | 6·5 | ·007 | ·009 | ·013 |
| 12″+ | | 0·45 | 0·05 | 286 | Nil | 6·6 | ·004 | ·009 | ·011 |
| S6 0—6″ | E2 | 3·35 | 0·31 | 114 | Trace | 6·7 | ·024 | ·010 | ·023 |
| 6—12″ | | 1·70 | 0·18 | 198 | Trace | 7·0 | ·006 | ·008 | ·013 |
| 12″+ | | 0·25 | 0·03 | 244 | Nil | 7·1 | ·003 | ·005 | ·011 |
| S7 0—6″ | B2 | 3·27 | 0·34 | 100 | 0·3 | 6·6 | ·029 | ·010 | ·034 |
| 6—12″ | | 1·29 | 0·13 | 190 | 0·2 | 7·3 | ·011 | ·008 | ·018 |
| 12″+ | | 0·56 | 0·06 | 232 | Nil | 7·1 | ·008 | ·007 | ·017 |
| S8 0—6″ | D2 | 3·44 | 0·34 | 108 | Nil | 6·7 | ·026 | ·009 | ·022 |
| 6—12″ | | 1·38 | 0·13 | 272 | Nil | 7·5 | ·007 | ·006 | ·011 |
| 12″+ | | 0·51 | 0·05 | 385 | Nil | 7·2 | ·005 | ·004 | ·011 |
| S9 0—6″ | A2 | 2·97 | 0·28 | 90 | 0·2 | 7·0 | ·028 | ·010 | ·031 |
| 6—12″ | | 1·75 | 0·17 | 238 | Nil | 7·0 | ·010 | ·007 | ·016 |
| 12″+ | | 0·42 | 0·04 | 306 | Nil | 6·7 | ·007 | ·007 | ·012 |
| S10 0—6″ | C2 | 3·11 | 0·30 | 96 | 6·7 | 7·7 | ·024 | ·011 | ·032 |
| 6—12″ | | 1·61 | 0·16 | 164 | 2·9 | 7·7 | ·009 | ·010 | ·011 |
| 12″+ | | 0·40 | 0·04 | 288 | Trace | 7·6 | ·005 | ·006 | ·011 |

The organic matter content is medium, falling off rapidly below 12″, and is lowest on plots containing free calcium carbonate. The latter varies from nil to 6·7%.

The exchangeable potassium is medium in the top 6″ but on plots D1 and B1 falls off rapidly with depth. Trees on these two plots suffered from potassium deficiency in the early years after planting. Exchangeable magnesium is generally low. This accounts for the low content of magnesium in the leaves. Citric soluble $P_2O_5$ is medium in the top 6″ but falls off rapidly with increase in depth.

The pH of the top soils varies from 5·7 to 7·8, the higher pH being associated with the presence of free calcium carbonate.

Easily reducible manganese was determined by hydroquinone in neutral ammonium acetate. The values for the top soils (0—6″) are bordering on the critical level (100 p.p.m.). At lower depths the values are adequate. Trees on plots with a high pH showed symptoms of manganese deficiency.

**Yield of Fruit.**

Treatments to date have had no significant effect on yield of fruit. In progress report I it was stated that treatments had a significant effect on yield of fruit in 1946. This interpretation was due to the incorrect treatment of the analysis of variance. Re-calculation showed that there was no significant difference due to cover crops.

Rootstocks had a significant effect on the yield of fruit from Dabinett in 1946, and from Stoke Red in 1948. In both cases the yield of fruit was greater from trees on crab rootstock. These varieties have shown a tendency to biennial bearing, so the yields for 1947 and 1948 were combined and re-analysed. For both varieties the yield from trees on crab was greater at the 5% level of P than from trees on Malling I. Rootstock had no significant effect on the combined yield of fruit from Sweet Alford.

**Girth Increase 1946-48.**

| | |
|---|---|
| Var. *Sweet Alford.* | No significant effect due to cover crops. |
| | Crab > Malling I. |
| Var. *Stoke Red.* | No significant effect due to cover crops. |
| | Crab > Malling I. |
| Var. *Dabinett.* | Cover crop effect significant at 5% level of P. |
| | Treatment B (Clover) > A, C and D. |
| | Crab > Malling I. |

## LEAF NUTRIENT STATUS.

**Sampling.**

In 1947 leaf samples were taken on 28—30th June, 1st August and 2nd September, in order to follow the changes in nutrient status during this period, and to obtain information on the best sampling time. Mature leaves from current terminal growths were taken in all cases. About one hundred leaves constituted a sample. The samples were oven dried, milled and analysed for total N, $P_2O_5$, $K_2O$, MgO and CaO.

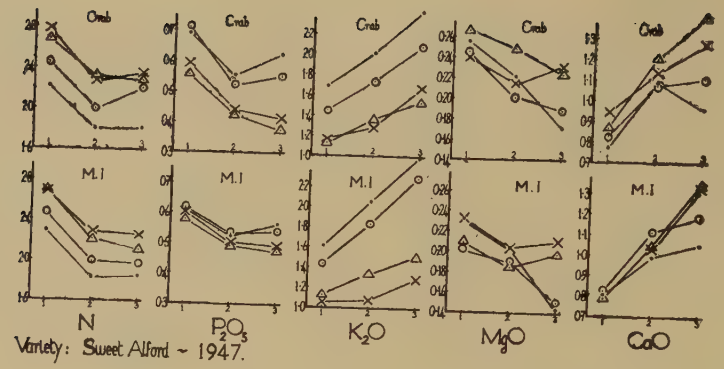
**Results.**

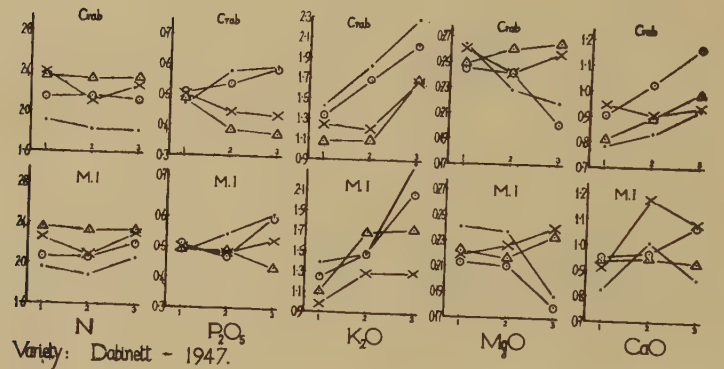
The results for 1947 are illustrated in *Fig.* 1, and the details from the third sampling only are given in Table II. It will be seen from *Fig.* 1 that differences due to treatments reach a maximum at the third sampling. Therefore in 1948, in order to reduce the analytical work, samples were taken on one date only, 26.8.48. The analytical data for 1948 leaf samples are given in Table II.

*Fig.* 1 illustrates very clearly the varietal differences in response to treatments. In 1947 the differences in nutrient status due to treatments were greatest in Sweet Alford, intermediate in Dabinett and least in Stoke Red. However, in 1948 the position was reversed, Stoke Red now showing the greatest differences in nutrient status due to treatments and Sweet Alford the least. In 1947 Stoke Red carried a very light crop but in 1948 the crop was approximately ten times greater.

*Nitrogen.*

1947. Cover crops had no significant effect on the nitrogen content of the leaf. The nitrogen content of leaves from trees growing in perennial rye grass was lower than that of leaves from trees growing under treatments B, C and D. 1948 treatments had a significant effect on the nitrogen content of leaves from all three varieties, the effect being more marked on Dabinett



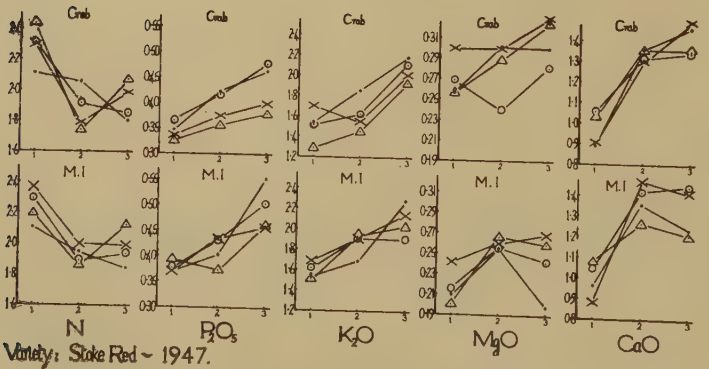




*Fig.* 1. Showing the effect of cover crops and time of sampling on the chemical composition of the leaf. All constituents expressed on a dry matter basis.

*Key*: Treatments: • = Perennial rye grass. o = White clover.
× = Annual cover crops. Δ = Clean cultivation.
*Sampling dates*: 1 = 30–6–47; 2= 1–8–47: 3 = 1–9–47.

and Stoke Red than on Sweet Alford. In all cases the nitrogen content of leaves from trees receiving treatments A and B was lower than that of leaves from trees receiving treatment C and D. The nitrogen content of leaves from Dabinett and Stoke Red under perennial rye grass (A) has fallen to deficiency level. The effect of clover appears to be intermediate between clean cultivation and perennial rye. It must be pointed out, however, that the proportion of clover on treatment B has greatly diminished since seeding. Rootstocks had no significant effect on the nitrogen content of leaves.

*Phosphorus.*

1947. Cover crops had a significant effect on the $P_2O_5$ content of Sweet Alford and Dabinett leaves, treatments A and B > C and D. With Stoke Red rootstock only had a significant effect, Malling I > crab.

1948. Significant effects were obtained with Stoke Red only. Treatments A > C and D. Malling I > crab.

*Potassium.*

1947. Differences due to cover crops were highly significant on Sweet Alford, approached significance on Dabinett, but were not significant on Stoke Red. With Sweet Alford treatments A and B > C and D. Rootstocks had no significant effect.

1948. Significant effects due to cover crops on all varieties. In all cases A > B, C and D. The differences between A, and C and D was most marked, in some cases approaching 100%. There was a marked difference between the effect of grass and annual cover crops in mobilising soil potassium. Rootstocks had no significant effect.

*Magnesium.*

1947. Cover crop effect not significant. Rootstock effect significant. Crab > Malling I.

1948. As for 1947. This effect of rootstock was very noticeable in the field, trees on Malling I suffering much more from magnesium deficiency than trees on crab. The magnesium status of leaves on all plots was at deficiency level. The inverse relationship between potassium and magnesium contents, and the direct relationship between low nitrogen and low magnesium can be seen from Table II.

*Calcium.*

No significant effect due to cover crops or rootstocks.

*Manganese.*

Owing to insufficient material, manganese was not determined on all samples. The range at the 3rd sampling in 1947 varied from 16 to 34 p.p.m. for Sweet Alford, and from 15 to 35 p.p.m. for Stoke Red. The critical value for apple leaves at this time of the season is in the region of 20 p.p.m. on a dry matter basis.

## DEFICIENCY SYMPTOMS.

A summary of the deficiency symptoms for 1948 is given in Table III. It will be seen that trees growing on grass plots showed no signs of potassium deficiency, whereas trees on plots C (annual cover crops) and D (clean cultivation) showed some potash scorch varying in severity from a trace to severe. On the other hand magnesium deficiency was least on plots C and D and greatest on plot A. Manganese deficiency was very localised and seemed to be associated with soil conditions rather than with treatments.

TABLE II.

EFFECT OF COVER CROPS ON THE CHEMICAL COMPOSITION OF APPLE LEAVES. ALL CONSTITUENTS EXPRESSED AS A PERCENTAGE OF DRY MATTER.

| Variety and Constituent | A Perennial Rye Grass | B White Clover | C Annual Cover Crop | D Clean Cultivation | Differences due to :— Cover Crop | Differences due to :— Rootstock | Sig. difference 1% | Sig. difference 5% | Remarks on Treatments |
|---|---|---|---|---|---|---|---|---|---|
| *Sweet Alford.* 1–9–47 | | | | | | | | | |
| Total N .. .. | 1·84 | 2·09 | 2·30 | 2·20 | N.S. | N.S. | | | |
| „ $P_2O_5$ .. | 0·599 | 0·555 | 0·453 | 0·426 | † | N.S. | 0·112 | 0·061 | † A > C and D; B > D. |
| „ $K_2O$ .. | 2·46 | 2·19 | 1·48 | 1·53 | † | N.S. | 0·619 | 0·337 | † A and B > C and D. |
| „ MgO .. | 0·158 | 0·171 | 0·221 | 0·211 | N.S. | * | | | * Crab > M.I. |
| „ CaO .. | 1·00 | 1·00 | 1·01 | 0·99 | N.S. | N.S. | | | |
| *Sweet Alford.* 26–8–48 | | | | | | | | | |
| Total N .. .. | 1·67 | 1·82 | 2·11 | 2·12 | * | N.S. | | 0·212 | * C and D > A and B. |
| „ $P_2O_5$ .. | 0·646 | 0·522 | 0·354 | 0·342 | N.S. | N.S. | | | |
| „ $K_2O$ .. | 2·248 | 1·910 | 0·902 | 1·251 | * | N.S. | | 0·699 | * A > C and D, B > C, |
| „ MgO .. | 0·187 | 0·203 | 0·272 | 0·269 | * | * | | 0·063 | * C and D > A and B. Crab > M.I. |
| *Stoke Red.* 2–9–47 | | | | | | | | | |
| Total N .. .. | 1·82 | 1·89 | 1·98 | 2·10 | N.S. | N.S. | | | |
| „ $P_2O_5$ .. | 0·508 | 0·487 | 0·427 | 0·420 | N.S. | † | | | † M.I. > Crab. |
| „ $K_2O$ .. | 2·24 | 2·00 | 2·09 | 1·98 | N.S. | N.S. | | | |
| „ MgO .. | 0·248 | 0·262 | 0·298 | 0·292 | N.S. | † | | | † Crab > M.I. |
| „ CaO .. | 1·35 | 1·39 | 1·45 | 1·28 | N.S. | N.S. | | | |

† Significant at 1% level of P.
* Significant at 5% level of P.

TABLE II—(Contd.).

EFFECT OF COVER CROPS ON THE CHEMICAL COMPOSITION OF APPLE LEAVES. ALL CONSTITUENTS EXPRESSED AS A PERCENTAGE OF DRY MATTER.

| Variety and Constituent | A Perennial Rye Grass | B White Clover | C Annual Cover Crop | D Clean Cultivation | Differences due to :— Cover Crop | Differences due to :— Rootstock | Sig. difference 1% | Sig. difference 5% | Remarks on Treatments |
|---|---|---|---|---|---|---|---|---|---|
| *Stoke Red.* 26–8–48 | | | | | | | | | |
| Total N .. .. | 1·74 | 1·88 | 1·98 | 1·95 | † | N.S. | 0·123 | 0·067 | † B, C and D > A.<br>* C and D > A and B; B > A |
| „ $P_2O_5$ .. | 0·476 | 0·392 | 0·323 | 0·318 | † | † | 0·102 | 0·056 | † A > C and D. M.I. > Crab. |
| „ $K_2O$ .. | 2·37 | 1·80 | 1·79 | 1·24 | * | N.S. | | 0·502 | * A > B, C, and D.<br>B and C > D. |
| „ MgO .. | 0·303 | 0·326 | 0·408 | 0·371 | N.S. | † | 0·019 | | † Crab > M.I. |
| *Dabinett.* 1–9–47 | | | | | | | | | |
| Total N .. .. | 1·95 | 2·15 | 2·29 | 2·36 | N.S. | N.S. | | | |
| „ $P_2O_5$ .. | 0·603 | 0·597 | 0·481 | 0·406 | * | N.S. | | 0·098 | * A and B > C and D. |
| „ $K_2O$ .. | 2·34 | 2·06 | 1·48 | 1·70 | N.S. | N.S. | | | |
| „ MgO .. | 0·204 | 0·194 | 0·248 | 0·250 | N.S. | * | | | * Crab > M.I. |
| „ CaO .. | 0·89 | 1·12 | 1·01 | 0·96 | N.S. | N.S. | | | |
| *Dabinett.* 26–8–48 | | | | | | | | | |
| Total N .. .. | 1·61 | 1·82 | 2·14 | 2·06 | † | N.S. | 0·297 | 0·162 | † C > B and A; D > A<br>* B > A. |
| „ $P_2O_5$ .. | 0·590 | 0·515 | 0·412 | 0·397 | N.S. | N.S. | | | |
| „ $K_2O$ .. | 2·41 | 1·92 | 1·25 | 1·50 | † | N.S. | 0·338 | 0·184 | † A > B, C and D; B > C & D.<br>* D > C. |
| „ MgO .. | 0·242 | 0·254 | 0·244 | 0·275 | N.S. | * | | | * Crab > M.I. |

† Significant at 1% level of P.
* Significant at 5% level of P.

TABLE III.

EFFECT OF TREATMENTS ON DEFICIENCY SYMPTOMS. RECORDED SEPT. 6-8th, 1948.

| Variety and Stock | A Perennial Rye Grass | | | | B Clover | | | | C Annual Cover Crops | | | | D Clean Cultivation | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N | K | Mg | Mn | N | K | Mg | Mn | N | K | Mg | Mn | N | K | Mg | Mn |
| *Sweet Alford* | | | | | | | | | | | | | | | | |
| Crab .. .. | M-G | — | T | T | G | — | T | — | G | SL | — | T | G | T | — | T |
| M.I. .. .. | M-G | — | M-S | M | G | — | SL | T | G | SL-M | — | T | G | T | T | T |
| *Stoke Red* | | | | | | | | | | | | | | | | |
| Crab .. .. | G | — | T | T | G | — | T | — | G | — | — | — | G | T | T | T |
| M.I. .. .. | G | — | T | T | G | — | T | — | G | T | T | — | G | T | T | T |
| *Dabinett* | | | | | | | | | | | | | | | | |
| Crab .. .. | M-G | — | — | — | G | T | T | — | G | M | — | — | G | SL-M | — | — |
| M.I. .. .. | M-G | — | — | — | G | T | T | — | G | M-S | — | — | G | M | — | — |

Key to deficiency categories :—

Nitrogen (N) — M = Medium green. G = Green.

Potassium (K), Magnesium (Mg), Manganese (Mn) — T = Trace. SL = Slight. M = Moderate. S = Severe.

The differences between varieties in their susceptibility to nutrient deficiencies is most marked. Sweet Alford appears to be most sensitive to magnesium and manganese deficiency, whilst Dabinett seems most sensitive to potassium deficiency. The deficiency patterns on Stoke Red are not so clearly differentiated as on the other two varieties.

## DISCUSSION.

It is too early to draw conclusions from this experiment, but the data so far obtained indicate that the treatments employed have had a marked effect on the nutrient status of the trees. The absence of any significant effect on the yield of fruit may be due partly to the fact that the trees had been grown for ten years prior to the present treatments, which have only been applied for three seasons.

The effect of the cover crop treatments on the potassium and phosphorus content of the leaf is most striking, being greatest when the cover crop is grass. The annual cover crops used have had little effect, the results being much the same as for clean cultivation. Sufficient evidence is not yet available to show how the grass cover crop increases the potassium and phosphorus content of the tree. It is known that grasses are good potassium feeders and may mobilise sources of potassium not available to fruit trees. The frequent cutting of the grass, leaving it to decay *in situ*, results in a steady turnover of available potassium.

It would appear that three cwts. of nitro-chalk per annum has not been sufficient to maintain an adequate nitrogen status in the trees on these plots during the early years after grassing down. It is known, however, that after a number of years of grass treatment nitrogen status often improves and the requirements of trees for nitrogen fertilizers are lowered. The present dressing of three cwts. per acre will therefore be maintained unless the trees continue to show signs of severe nitrogen deficiency. To increase the dressings at present above this rate on the clean cultivated plots would also probably result in too high a nitrogen status with detrimental effects on cider quality.

It is apparent from the Stoke Red results that cropping and leaf nutrient differences are closely related, and that a number of years must elapse before a clear picture of the effect of treatments on tree nutrition can be obtained.

## SUMMARY.

1. The effect of cover crops on yield, girth increase, leaf nutrient status and deficiency symptoms of three cider apple varieties is described.

2. Treatments had no significant effect on crop yield during 1946 to 1948. Yield of fruit from trees on crab rootstock was greater than from trees on Malling I for Dabinett during 1947, and for Dabinett and Stoke Red for the combined years 1947-48.

3. With the exception of the variety Dabinett, treatments had no significant effect on girth increase over the years 1946-48. In all cases the girth increase of trees on crab rootstock was greater than for trees on Malling I.

4. Treatments had a significant effect on leaf nutrient status. The effect of grass, as compared with clean cultivation, was to increase the percentage of potassium and phosphorus, and to decrease the nitrogen content. Annual cover crops had no significant effect on leaf nutrient status as compared with clean cultivation. The effect of white clover was intermediate between that of grass and clean cultivation.

5. Deficiency symptoms were related to treatments and nutrient status. Grass decreased the incidence of potassium deficiency leaf scorch and increased magnesium deficiency.

6. The botanical composition changed considerably during the course of the experiment.

## ACKNOWLEDGMENTS.

We wish to express our thanks to Miss P. Scadding for help with the analytical work.

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# NUTRIENT PLACEMENT IN RELATION TO FRUIT TREE NUTRITION.

# I. PROBLEMS AND METHODS.

By C. BOULD.

## INTRODUCTION.

The problems connected with nutrient placement in relation to fruit trees are more complex than those associated with shallow-rooting crops such as cereals and vegetables. Fruit trees are perennials, with extensive and deep root systems. These two facts must therefore be taken into consideration when deciding upon the method and the time of application of nutrients. One must know when the nutrients are required in order to influence growth and fruit bud differentiation, and also the time lag between application of nutrients and their utilization by the tree. This in turn involves a knowledge of soil properties and in particular the effect of soil on the availability and movement of plant nutrients. Failure to appreciate these aspects of tree nutrition often accounts for the absence of response to nutrient application. The object of this paper is to present some of the problems involved in the application and utilization of nutrients under orchard conditions, and to outline some of the methods used or suggested to overcome them.

## SOIL EFFECTS.

### Base-exchange.

Way (18) in 1850 first demonstrated the effect of soil on the movement of soluble salts. He showed that when a solution of potassium chloride was passed through a column of soil, the potassium replaced calcium in equivalent amount. This soil property, known as base-exchange, plays an important part in the downward movement of such nutrients as potassium, magnesium and calcium, and explains why, on heavy soils, it takes some considerable time for bases applied to the surface to penetrate into the subsoil layers. The tenacity

with which these cations (bases) are held by the soil colloids, and therefore the ease with which they are removed by the roots, varies with the nature of the cation. According to Cooper *et al* (5) the intensity of removal of cations from soil colloids by electro-dialysis varies in the order $K > Na > Ca > Mg$, and this series agrees with the ease of removal and rate of absorption of the cations by plants. Thus plants will absorb potassium much more readily than magnesium. It follows from these facts that small amounts of magnesium applied to a soil rich in exchangeable potassium may fail to influence the uptake of magnesium by the tree for two reasons. Penetration will be limited, due to base-exchange, and the high K/Mg ratio will favour the absorption of potassium. In order to increase the absorption of magnesium it may thus be necessary to concentrate the supply in restricted areas, preferably in the area of active absorbing roots.

For some time it was considered that only cations were adsorbed by soil colloids, but it is now recognised that certain anions such as phosphate enter into exchange reactions, thus anion exchange also plays a part in the movement of phosphate in the soil.

**Fixation.**

Soluble nutrients when added to the soil may be rendered insoluble by various processes in addition to exchange reactions. Fixation may be brought about by chemical or biological processes. If chemical, it may be a precipitation such as takes place when a soluble phosphate is added. The composition and availability to the plant of the precipitate varies according to the pH of the soil. Thus under neutral or alkaline conditions the phosphate will normally be precipitated as an insoluble calcium phosphate, whereas under acid conditions iron and aluminium phosphates may be formed. In either case the soluble phosphate is rendered insoluble, thus preventing further movement in the soil. Under many conditions in practice penetration of phosphate applied on the surface is limited to the top few inches of soil.

Potassium ions are adsorbed by the soil colloids and as such are available for plant growth. After a time, however, depending on the type of colloid and soil conditions, some of the potassium migrates into the clay lattice and becomes fixed in a non-exchangeable form which is only slowly available for plant growth.

Biological fixation may also account for the locking up of certain nutrients such as nitrogen, phosphorus, manganese, copper and zinc. This is due to the soil micro-organisms utilizing these nutrients for their own metabolism and so rendering them temporarily unavailable for plant growth. Nitrogen is immobilised by soil organisms particularly in the presence of carbohydrate material. The nitrate ion, unlike the ammonium ion, is not adsorbed by the soil and can penetrate quite rapidly before any biological fixation occurs.

Plant nutrients may also be rendered unavailable by oxidation. This applies particularly to manganese which is more available in the reduced state.

From this brief account of factors which may restrict the movement of nutrients in the soil, it will be realised that the application of soluble nutrients to the soil surface will not necessarily be followed by the absorption of those nutrients by the tree. A description will now be given of certain methods of applying nutrients to soils so as to minimise these adverse soil factors, and of methods designed to by-pass the soil by introducing the nutrients through the aerial parts of the tree.

**Soil Methods.**

In order to minimise the percentage of a nutrient fixed by the soil it is sometimes applied in localised areas, for example in a circle under the drip area of the branches. Rogers (15) has shown that the roots extend further than the branches, and that the maximum density of feeding roots is likely to occur at some distance from the trunk. By localising the application of a nutrient the fixing power of the soil within that area becomes saturated and a greater percentage of the nutrient remains available for absorption than if the same amount of nutrient was distributed over a larger area.

On cultivated soils the fertilizer may be applied prior to ploughing, or an attachment may be fitted to the plough to deposit the fertilizer in the bottom of the furrow. Both methods increase depth of penetration.

Another method which has been shown to increase the penetration of both potassium and phosphate is to apply them in conjunction with farmyard manure or some form of organic mulch (17).

Some workers have advocated the localisation of fertilizers in the form of briquettes. Their preparation and use has been described by Wilde and Wittenkamp (19).

Quite a different approach to nutrient placement is sub-soil injection of concentrated solutions. The nutrient, or mixture of nutrients, is dissolved in water in concentrations varying from 5 to 20% and is injected under pressure at varying depths depending on the type of crop and the nature of the soil. McKinnon and Lilleland (11) used this method for the treatment of potassium deficiency in prune trees. A study of the root system revealed most of the roots in the second, third and fourth feet of soil. Laboratory and field studies showed that, owing to the high potassium fixing power of the soil, surface applications at ordinary commercial rates could not be expected to influence the potassium content in the major root zone. By sub-soil injection they were able to increase the available potassium in the 2 to 4 ft. region. They state that the fixation of various cations and phosphate by base-exchange and precipitation may be such that surface application of fertilizers may never effect any chemical change in the mass of soil in which the major portion of the tree roots are found.

The injection method has also been described by Jouis (10), Bryner (4) and Glaenzer (8). Jouis made his injections at 35 cms. below the soil surface. He found that the area of diffusion was limited, and when one to three litres per hole were injected the spread in the horizontal direction varied from 8·5 to 12·2 cms. Among some of the advantages claimed by the authors for this method are :—(1) speed and convenience in handling, (2) localisation of heavy doses, (3) precision as to amount given, (4) economy of nutrients and (5) placement of nutrients in contact with the roots so that fixation by the soil is reduced to a minimum. The method is used for the routine application of fertilizers in some French orchards.

Demolon and Bastisse (6) have used the sub-soil injection technique for the treatment of lime-induced chlorosis in fruit trees by injecting pseudo-solutions of ferri-silicate. They claim that these pseudo-solutions are stable over a wide pH range and that the iron is not precipitated by calcium carbonate.

Irish (9) and Friend (7) describe methods of introducing solid nutrients below the soil surface, but the technique does not appear so attractive as the solution method.

## INTRODUCTION OF NUTRIENTS THROUGH THE AERIAL PARTS OF THE TREE.

### Solid Injection.

It is very difficult to correct certain deficiencies by soil treatment. This is the case with lime-induced iron chlorosis in fruit trees. As a result of the work of Bennett (1) and others it was shown that the chlorosis could be overcome by the injection of iron salts into the trunk or branches of the tree. The method is now in general use for the treatment of iron deficiency in apples, pears and plums in this country, and can also be used for the introduction of minor elements such as copper, zinc, boron and manganese. It is not suitable for nutrients required in larger amounts, such as potassium and magnesium.

### Liquid Injection.

This method is more useful for diagnostic than curative purposes. Its development and uses have been described in detail by Roach (12) and Roach and Roberts (13).

### Foliage Sprays.

This method of introducing nutrients into the plant is becoming more widespread. It is suitable for such nutrients as potassium, magnesium, iron, manganese, zinc, copper and boron. Nitrogen, in the form of urea, has also been employed by Boynton (3) and others on fruit trees.

At one time it was thought that the epidermis of apple leaves was covered with an impervious layer of cutin. A re-examination of leaf structure by Roberts *et al* (14) showed that the cutinised areas are discontinuous. Pectinaceous substances are present in all the walls of the epidermal cells. These pectinaceous substances form a continuous path reaching from the outside of the leaf to the vein extensions. This accounts for the ability of the leaf to absorb nutrients from solutions applied to its surface.

This method of nutrient application has certain advantages over soil application. Since the nutrient is placed in close contact with the metabolic areas, the response to treatment is normally very rapid, and less nutrient is required than for soil treatment. However, the response may be less durable than that from some of the other methods and the treatment may have to be repeated annually.

It is essential that a suitable form and concentration be used for each nutrient, and an efficient wetting agent be employed.

The nutrient may sometimes be mixed with routine sprays without deleterious effect on either spray or plant, thus saving labour.

It is felt that this method, used in conjunction with soil treatment, has a useful place in tree nutrition.

## INDIRECT METHOD OF INFLUENCING TREE NUTRITION.

### Cover Crops.

As reported elsewhere (2) soil management has a significant effect on the nutrient status of fruit trees. Grass cover crops increase the availability and uptake of potassium and phosphorus, but compete with the tree for nitrogen. It has been shown by Wallace (16) that grassing down has a beneficial effect on lime-induced iron chlorosis.

## CONCLUSION.

It will be apparent from the brief survey given that the problems of fruit tree nutrition are numerous and complex. Because of the deep rooting habit of fruit trees the restrictions imposed upon the movement of nutrients by soil must be understood and taken into account when applying nutrients. The adverse factors may sometimes be overcome by placing the nutrient in direct contact with the roots by means of sub-soil injection, or the soil may be by-passed and the nutrient injected into the trunk, or be absorbed through the foliage. On the other hand it may be possible to mobilise natural reserves of nutrients in forms suitable for absorption by the trees by the use of cover crops and so to economise in fertilizers. In all investigations it is important that attention should be paid to changes in the nutrient status of the plant when measuring response to treatments. Increased knowledge of the relationship between chemical composition and yield, and of methods whereby the composition can be readily changed, will prove of great value.

The problems of nutrient placement, and the effect of cover crops on soil fertility and tree nutrition are being studied at Long Ashton, with particular emphasis in every case on the effect of treatment on the nutrient status of the tree.

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# REPORT ON THE USE OF FOLIAGE SPRAYS FOR THE CONTROL OF MAGNESIUM DEFICIENCY IN APPLES

By C. BOULD and J. TOLHURST.

## INTRODUCTION.

The use of foliage sprays for the correction of mineral deficiencies in plants, particularly perennial crops like fruit trees, is becoming more widespread. The method is based on the fact, now well established, that plants are capable of absorbing nutrients from solution through their leaves. Some absorption may also take place through the stems. The absorption of nutrients through the leaf is quite rapid and a response is obtained in a comparatively short time.

The correction of magnesium deficiency in fruit trees by soil treatment is a slow process. This has been shown by the experiments of Kidson *et al.* (3), Southwick and Smith (4), Boynton (1) (2), Wallace (5) and others. There are two main reasons for the slow response. Firstly, the penetration of nutrients such as potassium and magnesium through the soil is restricted by base-exchange properties, and secondly, it is well established that there is an antagonism between the absorption of potassium and magnesium. Trees on soils with a high potassium status, therefore, respond very slowly to soil application of magnesium.

Boynton (2) states that when the MgO content of apple leaves at midsummer is $> 0{\cdot}40\%$ trees rarely show symptoms of Mg deficiency. Between 0·4 and 0·25% MgO leaf blotch symptoms may appear, depending on nutritional status. Below 0·25% MgO leaf symptoms will appear on some branches regardless of other conditions. He states that application of Epsom salts, dolomite or kieserite has little effect during the first season, but that three sprays of a 2% solution of magnesium sulphate in water are effective in correcting the symptoms. The magnesium sulphate may be combined with fungicides but damage sometimes results. Because of the slowness of soil applications a combination of spray and soil treatment is recommended. Southwick and Smith (4) recommend three early sprays of a 2% solution of Epsom salts, either alone or mixed with routine pest sprays, as a temporary cure whilst waiting for soil applications to work.

Wallace (5) in a short account of trials carried out during the past ten years on the correction of magnesium deficiency in fruit trees concluded that the trials seemed to justify a recommendation that growers with acute problems should try the spraying method, using a 2% solution of Epsom salts plus a spreader, and giving four to six fortnightly sprays beginning at petal fall.

The present trials were conducted on apples at two outside centres, one in the Pershore district on the variety Cox's Orange Pippin, and the other at Norton-sub-Hamdon on Laxton's Superb. Both varieties were suffering from severe magnesium deficiency, in the first case associated with deficient water supply and in the second with low nitrogen. Both plantations were of bush trees under permanent grass.

## EXPERIMENTAL.

### Cox's Orange Pippin Orchard.

### 1946 Treatments.

The trees selected for this experiment were from an area in a bush plantation about 15 years old where the growth was stunted. Three rows of eight trees each were chosen for the spray treatment.

The object of the experiment was to test the effect of time of application and number of sprays on tree growth and magnesium content of the leaf. A 2% solution of calcined kieserite (magnesium sulphate 18% MgO), using Estol H as a wetter at 10 fluid ozs. per 100 gallons, was used throughout. The number of sprays and times of application are given in Table I.

TABLE I.

TIMES OF APPLICATION AND NUMBER OF SPRAYS GIVEN TO COX'S O.P. TREES 1946.

| Date | Row 1 | | Row 2 | | Row 3 | |
|---|---|---|---|---|---|---|
| | Trees 1–4 | Trees 5–8 | Trees 1–4 | Trees 5–8 | Trees 1–4 | Trees 5–8 |
| 1–5–46 | * | * | | | | |
| 15–5–46 | * | * | * | * | | |
| 29–5–46 | * | * | * | * | * | * |
| 13–6–46 | | * | * | * | * | * |
| 27–6–46 | | | * | | * | * |
| 10–7–46 | | | | | * | |

* Trees sprayed on these dates.

Nitro-chalk was applied to all trees at the rate of 3 cwts. p.a. on 15–6–46.

After treatment, the trees were scored for extension growth and magnesium deficiency symptoms.

**Results.**

*Extension Growth.*—Application of sprays early in the season produced more extension growth than late sprays.

*Deficiency Symptoms.*—All treatments decreased the incidence of leaf symptoms.

*Magnesium Status of the Leaf.*—The chemical composition of the leaves is shown in Table II.

TABLE II.

CHEMICAL COMPOSITION OF COX'S O.P. LEAVES. COMPOSITE SAMPLE FROM FOUR TREES TAKEN ON 7–9–46.

| Row | Tree Nos. | No. of Sprays | as % Dry Wt. | | |
|---|---|---|---|---|---|
| | | | MgO | $K_2O$ | CaO |
| 1 | 1—4 | 3 | 0·262 | 2·24 | 1·39 |
| 2 | 1—4 | 4 | 0·316 | 2·47 | 1·95 |
| 3 | 1—4 | 4 | 0·163 | 2·54 | 2·05 |
| 3 | 5—8 | 3 | 0·140 | 2·55 | 1·49 |
| | Controls | None | 0·122 | 2·72 | 1·71 |

Three early sprays starting at petal fall (Row 1, trees 1—4) were much more effective in increasing the magnesium content of the leaf than three or four sprays applied three weeks later (Row 3, trees 1—4 and 5—8). Four sprays at fortnightly intervals, beginning at petal fall, raised the magnesium status to a level approaching normal.

The potassium status of the leaf was high, indicating that previous potassium applications had probably helped to induce magnesium deficiency. As will be seen from Table IV the ratio of exchangeable potassium to exchangeable magnesium in the soil was high, a factor known to affect adversely the absorption of magnesium from the soil.

**1947 Treatments.**

The layout and object of the experiment were modified during 1947. The object now was to compare the relative effectiveness of soil application and foliage sprays, and to observe the effects of mixing the magnesium sulphate with the routine lime sulphur sprays. Another row of eight trees was included in the layout. Each treatment was applied to a group of four trees in duplicate. The plots were divided into two blocks and the treatments were randomised within the blocks.

Comparison of the magnesium content of leaves from control plots, one sprayed the previous year and the other unsprayed, showed that the residual effect of the previous spray on magnesium content was negligible.

The treatments and times of application are given in Table III.

TABLE III.

Treatments Applied to Cox's O.P. Trees, 1947.

| Treatments | 1% Solution * Kieserite | | 2% Solution Kieserite | | |
|---|---|---|---|---|---|
| | 1–5–47 | 23–5–47 | 11–6–47 | 24–6–47 | 14–7–47 |
| A. Control .. .. | | | | | |
| B. 5 lb. of Kieserite per tree. Soil application 1–5–47 | | | | | |
| C. Foliage sprays $MgSO_4$ only | * | * | * | * | |
| D. Foliage sprays. First two combined with lime sulphur sprays .. | * (Pink Bud) | * (Petal Fall) | * | * | * |

* Kieserite = 16·5% MgO as $MgSO_4$.

Estol H was used as a wetting agent at the rate of 10 fluid ozs. per 100 gallons. Lime sulphur was used at 1 : 80 at the pink bud stage, and 1 : 120 at petal fall. (Grower's recommendation).

The surface soil application was given in a band 15—18″ wide under the drip area of the branches.

Soil samples were taken at 0—9″ on a plot basis prior to application of Kieserite. The soil, according to Dr. Osmond, was a Pershore Heavy Sand with very free drainage. Analytical figures are given in Table IV.

The magnesium status of the soil was low and the exchangeable K/Mg ratio high, both factors being conducive to magnesium deficiency.

It is known that low nitrogen conditions adversely affect the absorption of magnesium. The whole experimental area was therefore given a dressing of ammonium sulphate at the rate of 3 cwts. per acre after taking the soil samples.

**Results.**

No damage was caused to the foliage by mixing the magnesium sulphate with the lime sulphur and so far as could be judged the fungicidal properties of the lime sulphur were not impaired.

TABLE IV.

Analysis of Soil Samples (0–9″) From Experimental Plots. Sampled 1–5–47.

| Plot (See Table III) | As % Air Dry Soil | | Exchangeable K/Mg Ratio | pH |
|---|---|---|---|---|
| | Exchangeable Mg | Exchangeable K | | |
| A1 | ·0055 | ·028 | 5·1 | 6·3 |
| A2 | ·0057 | ·019 | 3·4 | 6·5 |
| B1 | ·0078 | ·034 | 4·3 | 6·6 |
| B2 | ·0047 | ·020 | 4·2 | 5·9 |
| C1 | ·0062 | ·029 | 4·6 | 6·2 |
| C2 | ·0060 | ·028 | 4·7 | 6·4 |
| D1 | ·0067 | ·031 | 4·6 | 6·3 |
| D2 | ·0062 | ·025 | 4·1 | 6·2 |

A check was made on leaf symptoms on 14-7-47 and gave the following results :—

| *Plot* | *Treatment* | *Mg Deficiency* |
|---|---|---|
| A1 | Control | Slight to medium |
| A2 | ,, | Trace to slight |
| B1 | Soil application | Medium |
| B2 | ,, ,, | Trace |
| C1 | 4 foliage sprays | Clean |
| C2 | ,, ,, | Clean to trace |
| D1 | 5 foliage sprays | Clean |
| D2 | ,, ,, | Trace |

Unfortunately, a hot dry spell followed and many trees were severely defoliated owing to lack of water. This made further comparison of leaf symptoms impossible and restricted extension growth. Leaf samples were taken on 3-9-47 and analysed for total magnesium. The results are given in Table V.

TABLE V.

Magnesium Content of Cox's O.P. Leaves from the 1947 Experiment. Composite Leaf Samples from Four Trees 3–9–47. Total MgO as a Percentage of Dry Matter.

| A Control | B 5 lb. Kieserite per tree | C 4 Foliage sprays | D 5 Foliage sprays | S.E. Mean of 2 plots |
|---|---|---|---|---|
| 0·222 | 0·222 | 0·366 | 0·348 | ·0303 |

Treatment differences were not significant at the 5% level of P.

Soil application had no effect on the magnesium content of the leaf. Foliage sprays increased the percentage of magnesium, but the differences due to treatment just failed to reach significance at the 5% level of P. (F test).

**1948 Treatments.**

Treatments were slightly modified during 1948 as described below :—

A. Control.

B. No soil application this year.

C. No pink bud spray. First spray at 2% concentration at petal fall followed by further sprays on 14-5-48 and 1-6-48.

D. No pink bud spray. First spray at petal fall (4-5-48) and further sprays at 2% concentration on 14-5-48, 1-6-48 and 14-6-48. Magnesium sulphate sprays applied separately, not combined with lime sulphur as in the previous year.

**Results.**

During July a dry spell again resulted in severe defoliation and made a comparison of leaf symptoms impossible. It would appear that water supply in this area is one of the factors limiting growth. Sufficient leaves were obtained for chemical analyses on 12-8-48. The effect of treatments on magnesium content is shown in Table VI.

TABLE VI.

Magnesium Content of Cox's O.P. Leaves from the 1948 Experiment. Composite Leaf Samples from Four Trees 12–8–48. Total MgO as a Percentage of Dry Matter.

| A Control | B Soil application 1947 | C 3 Foliage sprays | D 4 Foliage sprays | S.E. Mean of 2 plots |
|---|---|---|---|---|
| 0·173 | 0·189 | 0·286 | 0·344 | ·0219 |

Treatment differences were significant at 5% level of P. Treatments C and D > A and B.

Soil application in the previous year had no significant effect on the magnesium content of the leaf. Foliage sprays increased the magnesium status of the leaf. The difference between three and four sprays was almost significant.

**Laxton's Superb Orchard.**

This is a commercial orchard of bush trees under permanent grass. When visited in September 1947 the leaves were showing a variety of symptoms varying from a severe marginal and interveinal chlorosis, with or without necrotic areas, to green leaves with internal necrotic areas, the typical magnesium " blotch " symptoms. The relationship between symptoms and chemical composition is brought out in Table VII and the types are illustrated in Plate 1.

TABLE VII.

Chemical Composition of Laxton's Superb Apple Leaves. Showing the Relationship Between Composition and Deficiency Symptoms. Leaves Taken from Non-Fruiting Spurs.

| Sampling date | Leaf symptoms | As % Dry Matter | | | |
|---|---|---|---|---|---|
| | | N | MgO | $K_2O$ | CaO |
| 17–9–47 | Internal " blotch " (d) .. | 1·632 | 0·109 | 2·044 | 1·511 |
| „ | Chlorotic type (a & b) .. | 1·486 | 0·129 | 1·813 | 2·044 |
| 30–8–48 | Internal " blotch " (d) .. | 2·00 | 0·122 | 1·787 | — |
| „ | Chlorotic type with some necrosis (c) .. .. | 1·78 | 0·126 | 1·712 | — |

It will be seen from Table VII that when the nitrogen content is low the typical symptom is a chlorosis with or without necrosis, whilst at a higher nitrogen level typical internal "blotch" symptoms predominate. The nitrogen level in the 1947 samples was lower because the leaves were physiologically older than in the 1948 samples.

**Layout 1948.**

The area where magnesium deficiency was most severe was divided into blocks of eight trees, each block being separated from its neighbours by buffer rows. Each group of eight trees received one treatment. There was no replication as the trial was intended to be mainly demonstrational, hence the use of groups instead of single trees. In order to obtain some idea of variability in the magnesium status between the blocks of trees, leaf samples from non-fruiting spurs were taken prior to treatment. The magnesium content is shown in Table VIII. It will be seen that the variation between blocks of trees was not very great and that the magnesium status was generally low.

TABLE VIII.

MAGNESIUM STATUS OF LAXTON'S SUPERB APPLE LEAVES. BEFORE AND AFTER TREATMENT.

| Treatments | MgO as % Dry Matter | | | |
|---|---|---|---|---|
| | Before treatment 19–5–48 | After treatment 30–8–48 | Response to treatment | Increase over control |
| Control .. .. | 0·252 | 0·202 | — 0·050 | — |
| 1 Foliage spray .. | 0·287 | 0·302 | + 0·015 | 0·100 |
| 2 „ sprays .. | 0·273 | 0·343 | + 0·070 | 0·141 |
| 3 „ „ .. | 0·260 | 0·493 | + 0·233 | 0·291 |
| 4 „ „ .. | 0·262 | 0·800 | + 0·538 | 0·508 |

**Treatments.**

Four treatments and a control were included in the trial. They were :—

Control — no foliage sprays.
1. One spray at petal fall (19-5-48).
2. Petal fall + one two weeks later.
3. „ „ + two at two weekly intervals.
4. „ „ + three at two weekly intervals.

The foliage sprays consisted of a 2% solution of kieserite (16% MgO) using sulphonated lorol as a spreader. Sufficient spray was used to wet all the leaves thoroughly. Weather conditions on spraying varied from dull and mild to sunny and hot.

No foliage damage was caused by the sprays.

**Results.**

*Foliage Symptoms.*—The effect of treatments on leaf symptoms is shown in Table IX.

The main symptom during 1948 was a severe chlorosis accompanied by interveinal necrosis beginning at the margins.

It will be seen from Table IX that one foliage spray at petal fall considerably reduced the incidence of magnesium deficiency symptoms. Two sprays reduced the symptoms to a trace, and three sprays resulted in almost complete control. The effect on the quality of the foliage was most striking.

TABLE IX.

Effect of Spray Treatments on Leaf Symptoms of Apple. Var. Laxton's Superb.

| Treatments | 17–8–48 | | 29–9–48 | |
|---|---|---|---|---|
| | Chlorosis | Blotch | Chlorosis | Blotch |
| Control .. | Medium—severe | Trace | * Severe | Trace |
| 1 spray .. | Slight—medium | Trace | Slight—medium | Trace |
| 2 sprays .. | Trace—slight | Trace | Trace—slight | Trace |
| 3 sprays .. | Nil—trace | Nil | Nil | Nil—trace |
| 4 sprays .. | Nil | Nil | Nil | Nil |

* Chlorosis at this stage was accompanied by much marginal interveinal necrosis.

*Fruit Quality.*—The effect of spray treatment on quality of fruit was also very marked. Fruits on trees receiving two or more sprays were larger, more highly coloured and of better flavour than fruits on control trees.

*Magnesium Status of Leaves.*—The effect of treatment on the magnesium content of the leaf is shown in Table VIII. It will be seen that two foliage sprays raised the magnesium content to a figure approaching normal, whilst three or more sprays raised the content above normal.

## DISCUSSION AND CONCLUSIONS.

Three main conclusions can be deduced from these experiments which have a bearing on the control of magnesium deficiency in apple trees. Firstly, that early foliage sprays are more effective than late sprays in raising the magnesium status of the leaf. Secondly, that three to four foliage sprays of a 2% solution of magnesium sulphate, beginning at petal fall and then at fortnightly intervals, are sufficient to raise the magnesium status of the leaf from a deficiency level to normal, and to suppress the development of magnesium deficiency symptoms. Thirdly, that over a period of two years soil application of magnesium sulphate, under the conditions described, failed to influence either the magnesium status of the leaf or the incidence of magnesium deficiency symptoms.

In the 1947 trial the mixing of the magnesium sulphate with lime sulphur caused no damage to foliage, nor had it any observed deleterious effect on the fungicidal properties of the spray. This procedure, however, needs further investigation before it can be generally recommended, as varieties may react differently. Calcium sulphate may be deposited and cause mechanical trouble unless the solution is stirred.

It is known that trees suffering from a deficiency, or state of nutrient un-balance, are susceptible to spray injury. On theoretical grounds, therefore, the incorporation of the deficient element in the fungicidal spray, provided it does not react to form an injurious chemical product, should decrease the susceptibility to spray injury in succeeding sprays. On economic grounds also the mixing of the deficient element with the routine sprays is to be recommended provided it does not interfere with normal working conditions.

The residual effect, if any, of foliage sprays on the magnesium status of the tree is not yet known. It is likely that some magnesium is stored in the tree. The magnesium content of the leaves at leaf fall should be greater and so contribute a small amount to the soil.

The importance of using a wetting agent such as Estol H or sulphonated lorol cannot be overstressed. In the absence of a wetting agent the solution will form drops on the leaf which on drying will concentrate and cause damage.

The foliage spray method is effective in correcting magnesium deficiency and should be regarded as a quick method to be used until such time as soil applications become effective. These may take two to three years depending on the climate, physical state of the soil, magnesium compound used and the potassium status of the soil.


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# THE RESOLUTION OF THE FACTORS IN SOIL ACIDITY.

## IV. THE RELATIVE EFFECTS OF ALUMINIUM AND MANGANESE TOXICITIES ON SOME FARM AND MARKET GARDEN CROPS (Cont.)

By E. J. HEWITT.

The previous paper in this series (3) described some of the effects of manganese and aluminium toxicities on certain farm and market garden crops. This paper presents further results obtained with these and other crops given below.

## EXPERIMENTAL.

The plants were grown in sand cultures with a single basal nutrient containing a low level of calcium (30 p.p.m., i.e. 1·5 m.e.q./litre) and somewhat low in phosphorus (20 p.p.m., i.e. 2 m.e.q./litre as $PO_4'''$). The additional manganese and aluminium were given as sulphates at 55 p.p.m. (2 m.e.q./litre) and 36 p.p.m. (4 m.e.q./litre) of each element respectively, either alone or in combination at each watering. The aluminium level was thus in excess of the equivalent phosphate supply. The solutions without aluminium had pH values of 6·2 to 6·7 ; those with aluminium had pH values about 4·0. The remainder of the technique was similar to that described in the preceding papers (1), (2), (3). The sand was not, however, leached with cold 2% hydrochloric acid for the work described here, as was done in 1947 (3), and the calcium supply in the nutrient solution was obtained by dilution of tap water in a large tank with the appropriate amount of rain water.

The crops used were sown direct in the sand or transplanted from standard

Plate I.

Range of symptoms associated with magnesium deficiency in apple leaves.
a, b, and c. Low nitrogen and low magnesium.
d. High nitrogen and low magnesium.

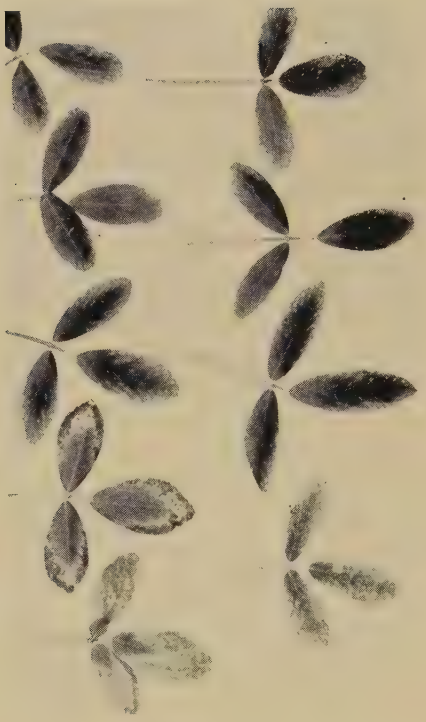

*Fig.* 1. Lucerne leaflets. Effects of manganese toxicity.

*Fig.* 2. Dwarf bean. Symptoms of manganese toxicity in petioles. Note reduced necrosis in leaf-base region.

*Fig.* 3. Marrow stem kale.
Left: Excess manganese.
Right: Excess aluminium.

*Fig.* 4. Celery.
Left: Excess manganese.
Right: Excess aluminium.

compost on 11-12/5/48 and included the following :—barley, oat, marrowstem kale, swede, cauliflower, Brussels sprout, tomato, potato, broad bean, dwarf bean, red clover, alsike clover, lucerne, sugar beet, mangold, linseed, carrot, celery, lettuce, leek. The basal nutrient was given at planting and the excess treatments were commenced about 10-14 days after sowing or transplanting. Some of the plants were given additional molybdenum (at 9·5 p.p.m. as molybdate) as well as manganese, but the results are not presented in detail at this stage.

## RESULTS.

The visual effects of manganese and aluminium toxicity are described in the appendix, except where they have been reported previously (1), (2), (3).

Marked calcium deficiency effects were seen in celery (5) grown in the basal and in the excess manganese treatments, also in potato during the initial period in all treatments (see (3)). Slight calcium deficiency was observed in tomato, broad bean and dwarf bean grown with the basal nutrient, and in alsike and red clover given excess of aluminium and excess of aluminium and manganese. These symptoms have been described elsewhere (4), (5), (7).

Symptoms of manganese toxicity were observed in all crops given excess of manganese, except leek. These symptoms were slight or barely perceptible in oat, sugar beet, mangold, broad bean and celery. Marked or severe symptoms appeared in the remainder. Those seen on crops used in earlier experiments confirmed previous descriptions. In general the effects of manganese toxicity were distinct from those known to be due to other mineral disorders. The type of necrosis produced in potato and barley, however, showed features analogous to effects of manganese deficiency (4), (7), whilst the symptoms in lucerne at one stage closely resembled potassium deficiency (4), though the potassium status was normal and similar to that of plants given the basal treatment, according to tissue test data. Some effects of manganese toxicity in lucerne are shown in Plate II *Fig.* 1. An interesting distribution of necrotic spotting occurred in the petiole region of dwarf bean. The petiole was speckled but the pulvinus regions were practically free from this effect, as shown in Plate II, *Fig.* 2.

The visual effects of excess of aluminium on many crops resembled symptoms of acute phosphorus deficiency. This was particularly so for carrot, celery, leek, barley, linseed, beet, mangold, dwarf bean, tomato, potato and red clover. Phosphorus deficiency was less marked in the Brassica group. Foliage symptoms apparently distinct from those associated with phosphorus deficiency were evident in alsike and lucerne. Root thickening and injury characteristic of aluminium toxicity were observed in sugar beet, mangold, barley, red clover and lucerne. The effects of aluminium toxicity were somewhat less severe than in 1947. This was attributed to the use of unleached sand containing some calcareous material, and confirmed by resowing barley and sugar beet in leached sand.

The combined effects of aluminium and manganese excess frequently accentuated or accelerated the onset of symptoms of manganese toxicity, as for example in the Brassica group, dwarf bean, clovers, potato and carrot. Extra manganese appeared to reduce or mask some of the phosphate deficiency effects (particularly the pigment formation) induced by excess of aluminium. This response was most noticeable in dwarf bean, red clover, tomato, potato and carrot. The simultaneous effects of phosphorus deficiency and manganese toxicity became confused later in the season and one or the other tended ultimately to predominate. Manganese toxicity was the dominant symptom in the

Brassicae, alsike clover, dwarf bean and potato; phosphorus deficiency or aluminium toxicity later became the dominant effect in carrot, celery, lucerne, red clover, linseed, broad bean and mangold. Both effects persisted in tomato and barley.

The order of relative susceptibility of different crops to manganese and aluminium toxicities varied considerably, as noted previously (3). Generally speaking aluminium caused more injury than manganese, but swede and kale were two outstanding exceptions. These crops were much less injured by excess of aluminium than by manganese, as noted in earlier work (3). Celery and leek were two crop plants, in addition to sugar beet, mangold and barley already noted (2), (3), that were far more susceptible to aluminium toxicity than to manganese toxicity. Examples of these relative effects are shown in Plate II, *Figs.* 3 and 4.

Yields were recorded although no test of significance could be made in this experiment. The yields of plants given excess of aluminium ranged from 2—5% of that for the basal treatment for celery and carrot to 50—60% for oat, potato, kale, swede, alsike and cauliflower. Relative yields for plants given excess of manganese ranged from 25—30% for swede and kale to 50—60% for potato and oat. Combined aluminium and manganese excess always depressed yield relative to manganese excess alone. Growth relative to that produced with aluminium excess alone was slightly increased in many crops regarded as being more susceptible to aluminium injury or induced phosphorus deficiency, but was depressed in those crops considered to be relatively sensitive to manganese excess e.g. Brassicae. The relative effects are given in Table I.

TABLE I.

Relative Yields of Crops given Excess Manganese and Aluminium Treatment as Percentage of Yield in Basal Treatment.

| Crop | Treatment | | |
|---|---|---|---|
| | Excess Manganese | Excess Aluminium | Excess Manganese and Aluminium |
| Swede .. .. | 25 | 50 | 5 |
| Kale .. .. .. | 30 | 60 | 12 |
| Tomato .. .. | 40 | 20 | < 25 S* |
| Lucerne .. .. | 40 | 25 | 6 |
| Sprout .. .. | 45 | 20 | 4 |
| Carrot .. .. | 50 | 6 | < 8 S |
| Linseed .. .. | 55 | 10 | < 14 S |
| Red Clover .. .. | 60 | 30 | 12 |
| Cauliflower .. .. | 60 | 60 | 16 |
| Celery .. .. .. | 65 | 2·5 | < 3·5 S |
| Barley .. .. | 70 | 25 | < 60 S |
| Leek .. .. .. | 70 | 8 | = 8 S |
| Sugar Beet .. .. | 85 | 15 | < 17 S |
| Mangold .. .. | 85 | 20 | 15 S |
| Potato (tubers) .. | 90 | 50 | 50 |
| Alsike .. .. .. | 100 | 50 | 45 |
| Oat .. .. .. | 100 | 50 | < 60 |

*S. Crops marked thus are considered to be particularly susceptible to aluminium toxicity and induced phosphorus deficiency.

< Indicates slight increase over aluminium only series.

TABLE II.

SUMMARY OF TISSUE TEST DATA ON STANDARD PETIOLE EXTRACTS (6). (FIGURES AS P.P.M.).

| Treatment | Kale 30.7.48 | | | Tomato 15.6.48 | | | Potato 15.6.48 | | | Carrot 8.7.48 | | | Celery 23.8.48 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ca | Mn | $PO_4$ | Ca | Mn | $PO_4$ | Ca | Mn | $PO_4$ | Ca | Mn | $PO_4$ | Ca | Mn | $PO_4$ |
| Basal .. .. .. | 40 | 0·2 | 30 | 80 | N.D.* | 5 | 110 | N.D. | 8 | 10 | 0·2 | 15 | 25 | 0·2 | 20 |
| Basal + Mn .. .. | 30 | 20 | 30 | 40 | 21 | 10 | 50 | 4·5 | 8 | 50 | 12 | 10 | 10 | 10 | 30 |
| Basal + Al .. | 30 | 0·2 | 10 | 80 | 0·5 | 3 | 50 | N.D. | 0 | 50 | 0·5 | N.D. | 25 | 0·2 | 20 |
| Basal + Mn + Al | 40 | 30 | 15 | 80 | 32 | 5 | 70 | 7·5 | 3 | 10 | 15 | 5 | 25 | 7·5 | 10 |

| Treatment | Dwarf Bean 15.6.48 | | | Broad Bean 30.7.48 | | | Red Clover 23.8.48 | | | Lucerne 8.7.48 | | | Sugar Beet 23.8.48 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ca | Mn | $PO_4$ | Ca | Mn | $PO_4$ | Ca | Mn | $PO_4$ | Ca | Mn | $PO_4$ | Ca | Mn | $PO_4$ |
| Basal .. .. .. | 57 | N.D. | 30 | 100 | 0·2 | 3 | 40 | 0·2 | 30 | 80 | N.D. | 45 | 25 | 0·2 | 30 |
| Basal + Mn .. .. | 100 | 5 | 25 | 50 | 12·5 | 3 | 50 | 30 | 40 | 20 | 5·5 | 35 | 10 | 4 | 30 |
| Basal + Al .. | 100 | N.D. | 5 | 50 | 0·2 | N.D. | 40 | 0·2 | 10 | 75 | 0·5 | 15 | 25 | 0·2 | 10 |
| Basal + Mn + Al | 50 | 40 | 20 | 40 | 15 | N.D. | 40 | 10 | 5 | 50 | 23·5 | 15 | 10 | 3 | 20 |

*(N.D. implies not detected).

TABLE III.

Distribution of Extractable Ca, Mn and $PO_4$ in Leaves of Brassica Crops. (Figures as p.p.m. in Standard Extract (6))

| Crop and Treatment | Petiole | | | Intervenal Areas | | | Margin (5 mm. strip) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ca | Mn | $PO_4$ | Ca | Mn | $PO_4$ | Ca | Mn | $PO_4$ |
| *Cauliflower* | | | | | | | | | |
| Basal .. .. .. | 40 | 0·2 | 45 | 75 | 0·5 | 25 | 100 | 0·5 | 25 |
| Basal + Mn .. .. | 30 | 10 | 15 | 50 | 17 | 20 | 75 | 30 | 25 |
| Basal + Al .. .. | 30 | 0·2 | 5 | 50 | 0·5 | 10 | 75 | 95 | 10 |
| Basal + Mn + Al .. | 40 | 10 | 12 | 75 | 25 | –* | 50 | 40 | –* |
| *Swede* | | | | | | | | | |
| Basal .. .. .. | 25 | 0·2 | 20 | 70 | 0·5 | 45 | 100 | 1·0 | 60 |
| Basal + Mn .. .. | 15 | 5 | 15 | 100 | 14 | 40 | 100 | 31 | 45 |
| Basal + Al .. .. | 30 | 0·2 | 5 | 100 | 0·5 | 15 | 110 | 2·5 | 20 |
| Basal + Mn + Al .. | 30 | 8·5 | 15 | 100 | 32 | 25 | 120 | 56 | 30 |
| *Brussels Sprout* | | | | | | | | | |
| Basal .. .. .. | 125 | 0·2 | 15 | 125 | 0·2 | 35 | 250 | 0·2 | 35 |
| Basal + Mn .. .. | 100 | 17·5 | 17 | 75 | 30 | 40 | 200 | 40 | 40 |
| Basal + Mn + Al .. | 125 | 15 | 5 | 100 | 45 | 10 | 125 | 60 | 15 |

* Not determined owing to insufficient sample.

Tissue tests were made at intervals in the season on a number of crops. The results confirmed that aluminium could not be extracted from foliage by the standard sodium acetate—acetic acid buffer. The amounts of manganese extracted from different crops varied considerably (Table II). There was, however, no clear correspondence between the manganese content so determined and susceptibility to injury from excess manganese. Additional aluminium usually increased the amount of extractable manganese, except in celery, clover, sugar beet and sprout. The soluble phosphate was reduced by aluminium but this reduction was partly offset in the presence of additional manganese in cauliflower, swede, kale, carrot, potato, tomato and dwarf bean, but not in red clover and celery (Tables II and III). The leaf cupping symptoms produced in Brassicae were associated with relatively high accumulation of manganese in the leaf margins (Table III) and confirmed previous findings (2). The marginal accumulation of manganese was usually accompanied also by an increase in the calcium content.

## DISCUSSION.

The results presented in this report will not be discussed in detail. A few points, however, call for comment at this stage. The correspondence

between the leaf cupping symptoms and site of manganese accumulation in Brassicae is of interest. The cupping may be due to reduction in relative growth rate caused by the local accumulation of manganese. The curious morphological demarcation of necrotic speckling in the petiole region of the dwarf bean leaf also provides opportunity for further investigation. The visual and tissue test relationship between the incidence of aluminium-induced phosphorus deficiency and the level of manganese given is noteworthy ; the slight improvement in growth noted in many crops when additional manganese was given together with the aluminium was consistent with the idea that phosphorus availability was greater. This point evidently requires confirmation and further investigation. The effect of additional aluminium on manganese accumulation agrees with the visual response but the specific effects of aluminium *per se* and of reduced pH cannot be distinguished in these experiments. The fact that the susceptibility of different crops to injury by excess of manganese or aluminium varies widely and independently further illustrates the complexity of the problems of acid soils and the responses of different crops grown on them.

## SUMMARY.

1. Twenty farm and market garden crops were grown in sand culture and the effects of excess of aluminium or manganese, or both, on growth were observed. Visual symptoms and the tissue test levels of certain elements were recorded.

2. Distinctive but diverse symptoms of manganese toxicity were recorded for most of the different crops.

3. Aluminium toxicity effects were more uniform and appeared to induce phosphorus deficiency symptoms in most crops, as well as characteristic root injury in a few.

4. Combined excess of both aluminium and manganese generally accentuated manganese toxicity symptoms and often slightly reduced the phosphorus deficiency effect due to the excess aluminium.

5. There was no clear relationship between the susceptibility to manganese toxicity and the manganese extractable from petioles. No appreciable aluminium could be extracted from foliage. The amount of phosphorus extracted from petioles was decreased when aluminium was given but was frequently slightly greater in the presence of manganese and aluminum together than when aluminium was given alone.

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# APPENDIX.

## Description of Visual Symptoms of Manganese and Aluminium Excess.

### Excess Manganese.

*Brussels Sprout.* Growth reduced; natural leaf cupping accentuated; foliage pale, marginal paling followed by brown necrotic spotting.

*Linseed.* Growth slightly reduced; foliage darker green, bronze or orange-brown rounded spotting irregularly on lower and mid-stem leaves, appearing early on cotyledons which later became orange tinted.

*Carrot.* Growth slightly reduced; foliage pale, older leaves paling, chlorosis of leaf margins followed by bronze necrotic speckling along leaf margins and scorching.

*Celery.* Death of growing point; blackening of younger leaves due to calcium deficiency; older leaves, marginal and slight intervenal paling followed by yellow or bronze tinting.

*Lettuce.* Growth reduced; foliage pale and dull yellow around leaf margins.

*Leek.* No symptoms.

*Broad bean.* Growth slightly reduced; yellowing or bronzing of leaf margins of older leaves, and diffusely intervenally.

*Dwarf bean.* Growth reduced; first symptoms brown-purple necrotic markings in minor veins of prophylls; older leaves bright pale-green diffuse intervenal mottling between major veins, becoming dull yellow or white blotchy mottle; brown markings in finest veins and fine speckling in areas between veins; younger leaves intervenal chlorosis, marginal distortion and waving; foliage becoming dark due to profuse dark brown or purple necrotic speckling and breakdown of minor veins; petioles and stems profuse purple necrotic speckling, *absent or nearly so from pulvinus regions at leaf base and bases of leaflets.* (Plate II, *Fig.* 2).

*Alsike.* Growth slightly reduced; leaf margins pale yellow green, intervenal buff blotching, slight marginal necrosis and intervenal hatching; upper leaflets slightly distorted, particularly along margins; flowering accelerated.

*Lucerne.* Growth reduced; marginal paling of mid-stem leaves, followed by pale brown or buff spotting near margins; younger leaves distorted at tips, wavy margins or twisting of lamina. Later two types of symptoms distinguished:—

(a) yellow-green or grey-green paling at leaf tips; margins becoming brown or bronzed and irregularly speckled.

(b) buff or pale brown speckling in clearly defined marginal zone, as in potassium deficiency, followed by marginal paling, browning and scorching.

### Excess Aluminium.

*Kale.* Growth slightly reduced; foliage dull green.

*Swede.* Growth slightly reduced; foliage dull, faint purple tints, disappearing later.

*Cauliflower.* Growth reduced; foliage dull green, purple tinting of older leaves, disappearing later.

*Brussels Sprout.* Growth reduced, stunted; foliage pale green, lustreless, older leaves pale red, purple tinting and bronzing.

*Tomato.* Growth reduced; older leaves dark green, purple tinting, leaflets curled under and leaves pointed down.

*Potato.* Growth reduced; foliage dull olive-green tint, upcurled marginal scorch of middle leaves.

*Mangold.* Growth greatly reduced (almost stationary); foliage dark dull green; roots brown, stunted, laterals blackened and enlarged at tips.

*Linseed.* Growth thin; lateral branching suppressed; leaves pointing up, foliage pale, dull olive-green.

*Carrot.* Growth severely dwarfed; foliage dull dark green, dark purple tints in cotyledons, around leaf margins and on petioles and crowns; roots dwarfed.

*Celery.* Growth normal at first, later severely dwarfed; foliage dull olive-green with intense purple tinting of older leaves followed by scorching and withering.

*Lettuce.* Growth slightly reduced, rosette habit; foliage dull green.

*Leek.* Growth reduced; foliage dark green and lustreless.

*Broad bean.* Growth thin and stunted; foliage dull green, older leaves olive green tint, later scorched; few pods; premature death.

*Dwarf bean.* Growth reduced and thin; foliage dull green, dark at first, becoming olive green; premature rapid defoliation; few flowers or pods.

*Red Clover.* Growth thin; foliage dull, dark blue-green, older leaves olive-green with irregular dark brown or purple spotting, finally scorching and withering, collapse of petiole; few flowers; roots coarse.

*Alsike.* Growth thin, reduced; foliage dull blue-green, later olive- or yellow-green, some upcurled scorching; roots normal.

*Lucerne.* Growth thin and dwarfed; foliage dull grey-green or blue-green, later olive-green with brown upcurled marginal scorch and occasional irregular dark spotting; few flowers; roots coarse and thickened.

## Excess Manganese and Aluminium.

*Kale.* Severe dwarfing; manganese toxicity greatly accentuated; severe necrosis of laminae of all leaves leaving only vascular parts; death of growing point; dark markings on petioles and stems. No symptoms of phosphorus deficiency.

*Swede.* Typical severe manganese toxicity, more severe than with manganese only. No symptoms of phosphorus deficiency.

*Cauliflower.* Growth severely dwarfed; severe intervenal necrosis, marginal browning of leaves; no phosphorus deficiency.

*Brussels Sprout.* Growth severely dwarfed; severe manganese toxicity effects and slight purple tinting of older leaves as in phosphorus deficiency.

*Tomato.* Manganese toxicity symptoms more severe; purple tinting reduced; much taller than plants given extra aluminium only.

*Potato.* Manganese toxicity effects more marked, especially petiole and stem speckling; phosphorus deficiency less pronounced than in plants given aluminium only.

*Mangold.* Growth severely dwarfed; foliage dull dark green but also showing faint marginal mottling due to manganese toxicity effects.

*Linseed.* Growth thin, unbranched habit; foliage dull green but also showing irregular spotting caused by manganese toxicity, leaf tips distorted; early defoliation.

*Carrot.* Similar to manganese toxicity effects in early stages; growth rapidly checked with development of dull dark green foliage as in aluminium toxicity but purple tinting less marked.

*Celery.* Growth dwarfed; foliage less dull green than in extra aluminium only, olive tint but no purpling.

*Lettuce.* Dwarfed, rosette habit; pale foliage with pronounced yellow rim effect, later withering of outer leaves.

*Leek.* As for extra aluminium only.

*Broad bean.* Growth habit as for aluminium only; symptoms of manganese toxicity more marked, foliage dull green and withering early.

*Dwarf bean.* Growth dwarfed; leaves small and distorted, severe manganese toxicity symptoms accentuated; profuse intervenal necrosis but less vascular necrosis; slight symptoms of phosphorus deficiency and defoliation.

*Red Clover.* Growth dwarfed and thin; initial symptoms of manganese toxicity accentuated, leaf cupping and marginal distortion severe, fine necrotic spotting producing bronzed effect; symptoms of manganese toxicity later masked by onset of phosphorus deficiency effects due to excess aluminium, but less acute than with aluminium only.

*Alsike.* Growth reduced; foliage pale green, leaf margins grey- or yellow-green, bronze tint, curled forward and cupped, pale brown irregular batching just within pale distorted marginal region; manganese toxicity effects far more severe; later foliage olive green, slight phosphorus deficiency but less acute than in extra aluminium only.

*Lucerne.* Growth severely dwarfed and thin; foliage bronze tinted, mid-stem leaves yellow-green, marginal paling commencing at leaf tip becoming broad orange-brown zone, followed by brown necrotic areas in marginal region and irregular necrotic speckling; younger leaves irregular chlorotic bleached areas, marginal and tip distortion; later slight phosphorus deficiency symptoms, foliage becoming blue-green, severe defoliation leaving bare stems.

# EXPERIMENTS ON IRON METABOLISM IN PLANTS
## I. SOME EFFECTS OF METAL-INDUCED IRON DEFICIENCY.

By E. J. HEWITT.

Iron deficiency in plants is generally recognised by the characteristic pattern of chlorosis produced mainly in the younger foliage. Spraying or painting chlorotic leaves with a dilute solution of ferrous sulphate and a wetting agent usually produces recovery and fresh chlorophyll formation. Such chlorosis, however, as has been pointed out by Wallace and Hewitt (24), is seldom due under field conditions to simple lack of iron.

Considerable attention has been given to the ability of manganese to induce chlorosis in water culture and this has been confirmed in different plants in several experiments reviewed recently by Twyman (23) and by Wallace and Hewitt (24). Several other metals, including copper, cobalt, chromium and zinc, may induce symptoms of iron deficiency in culture experiments, and in the field, either as the result of natural deposits of compounds of these metals, or where mine or smelting works dumps or the spreading of heavily contaminated industrial sewage have produced toxic concentrations of such metals in the soil (9), (24).

This paper presents the results of three experiments carried out between 1946 and 1948, on the subject of metal-induced iron deficiency and describes the visual effects of excesses of several metals on a number of crop plants. Analytical data are reserved for publication later.

## EXPERIMENTAL.

### Materials and Methods.

The plants were grown in sand culture in ten-inch clay pots suitably treated to prevent their causing contamination with iron or manganese. The sand was retained by glasswool and a watch glass over the drainage hole. The sand was a high grade silica sand, details regarding the composition and purification of which have already been given (5). Distilled water which contained 0·005 p.p.m. each of iron and manganese was used in the 1946 experiments. In the later work rainwater purified by the Permutit synthetic resin ion-exchange method (5) was used. This water contained *less* than 0·002 p.p.m. each of iron and manganese.

The basal nutrient solution used is shown below and the stock solutions were purified by the method of Stout and Arnon (22). Ferric citrate used in 1946 to supply iron was purified from manganese (12), (19), but still retained some manganese equal to ·0011 p.p.m. manganese per 1·0 p.p.m. of iron. Manganese sulphate was prepared free from iron by boiling the stock solution with a small quantity of sodium bicarbonate and then filtering.

Iron and manganese components were given as stated below for the various experiments. There were three pots in each treatment.

In the 1946 experiments oat (var. Early Miller) was sown on 18/3/46 and thinned to twenty plants per pot soon after emergence. The plants were harvested on 16/6/46 and the roots removed. The sand was then leached

Composition of Basal Nutrient Solution.

| Reagent | G. wt. per 100 L. | Milli equivalents per Litre (m.e.q./L.) |
|---|---|---|
| $Ca(NO_3)_2$ | 54·61 | 6·67 |
| $KNO_3$ | 30·33 | 3·33 |
| $NaH_2PO_4 2H_2O$ | 20·80 | 4·0 (as $PO_4'''$) |
| $MgSO_4\ 7H_2O$ | 36·80 | 3·0 |
| $CuSO_4\ 5H_2O$ | 0·025 | 0·002 |
| $ZnSO_4\ 7H_2O$ | 0·029 | 0·002 |
| $H_3BO_3$ | 0·186 | 0·03 (millimol) |
| $(NH_4)_6Mo_7O_{24} 4H_2O$ | 0·0035 | 0·0002 „ |
| | pH 5·9 approx. | |

prior to transplanting seedling sugar beet plants (4 per pot) on 30/6/46 ; these were harvested on 10/9/46. The treatment designations, concentrations of iron and manganese and the equivalent $Fe^{+++}/Mn^{++}$ ratios for these experiments are shown in Table I.

The 1947 experiment was made with sugar beet only, grown with toxic concentrations of several metallic ions given separately. Sugar beet seedlings (9 weeks old) were transplanted three per pot on 22/5/47 and arranged in two series of three pots per treatment as follows :—

Series 1 :—Purified sand only with iron supplied as ferric citrate at 0·1 m.e.q./L. in the complete nutrient solution.

Series 2 :—Purified sand intimately mixed with magnetite (ground to pass 100 mesh) at the rate of 100 p.p.m. but without any iron in the nutrient solution. These levels of iron were regarded as just sufficient for normal growth. In both series the ions $Pb^{++}$, $Zn^{++}$, $Cu^{++}$, $Co^{++}$, $Ni^{++}$, $Mn^{++}$, $Cr^{+++}$ and $CrO_4''$ (this last given to supply the same weight of chromium as in $Cr^{+++}$) were added separately at concentrations of 0·5 and 1·0 m.e.q./L. in terms of the molecular weight divided by the valency in which the metal was given (except for $CrO_4''$). There were three control treatments in which iron was supplied either as magnetite or citrate or was omitted altogether.

The methods used for the third experiment in 1948 were similar to those used in 1947. Iron was given as ferric citrate. The same metal ions at 0·5 m.e.q./L. concentration as defined above were given to tomato, potato, oat, kale and sugar beet and in addition those of $Ti^{+++}$, $VO_3'$, $AsO_4''$, $Cd^{++}$, $Sn^{++}$, $MoO_4''$, $Ce^{++++}$, $WO_4'$, $Tl^{++}$ and $Pb^{++}$ were given to sugar beet only. For sugar beet, the omitting iron, basal treatment and extra $Cu^{++}$ and $Zn^{++}$ treatments were repeated with urea instead of nitrate as a source of nitrogen, while the omitting iron treatment and the additions given in 1947 (except $Pb^{++}$) were repeated in the presence of extra $MoO_4''$. The plants were transplanted or sown 2-3/6/48 and given the basal treatment until 9/6/48.

Sugar beet was used on account of its susceptibility in the field to induced iron deficiency and its tolerance to high concentrations of manganese (7). Oat, potato, tomato and kale also show striking effects in the field in the presence of heavy metal excesses.

**Results.**

**1946 Experiment.**

Oat plants grown with treatments in which either iron or manganese, or both, were omitted were greatly reduced in growth and died prematurely without forming grain. Iron deficiency appeared in 14 days in all treatments where iron was omitted. Typical symptoms of manganese deficiency appeared in 23 days where this element was omitted. Simultaneous iron and manganese deficiency effects were produced in the $Fe_0$ $Mn_0$ treatment and appeared on the same leaf, as shown in Plate III, *Fig.* 1. Foliage was dull and pale green in the $Fe_1$ $Mn_0$ and $Fe_2$ $Mn_0$ treatments and showed intervenal paling. No paling occurred in the $Fe_3$ $Mn_0$ plants. Manganese deficiency symptoms were most severe in the $Fe_2$ $Mn_0$ treatment but improved in the $Fe_3$ $Mn_0$ treatment. Slight iron deficiency symptoms appeared in the $Fe_1$ $Mn_2$ and $Fe_1$ $Mn_3$ treatments, and were more marked in the latter, but the $Fe_0$ $Mn_1$, $Fe_0$ $Mn_2$ and $Fe_0$ $Mn_3$ sets appeared all equally severe.

The visual responses of the sugar beet were similar in many respects but certain differences were observed. No simultaneous iron and manganese deficiency appeared in the $Fe_0$ $Mn_0$ treatments where only severe manganese deficiency was produced. The order of severity was $Fe_0$ $Mn_0 < Fe_1$ $Mn_0 = Fe_2$ $Mn_0 > Fe_3$ $Mn_0$. This last treatment produced markedly better growth. The variation in iron deficiency symptoms was greater than with the oats. Severity was markedly increased by increased manganese level when iron was omitted and slight chlorosis also appeared in the $Fe_1$ $Mn_2$, $Fe_1$ $Mn_3$ and $Fe_2$ $Mn_3$ treatments. Relative differences in growth and incidence of chlorosis in sugar beet are shown in Table I.

**1947 Experiment.**

Plants in the control treatment where iron was omitted developed acute symptoms of chlorosis and also brown intervenal necrosis typical of iron deficiency. Control plants given iron, either as citrate or as magnetite, grew vigorously and the foliage was normal. Chlorosis of younger leaves, typical of iron deficiency, developed in all plants given the additional ions and ranged from very severe (total yellow) to a trace (faint fine pattern mottling). Brown intervenal necrosis usually developed in the chlorotic leaves, including those of control plants lacking iron. This necrosis ranged from very severe with $Co^{++}$ or $Ni^{++}$ to slight or nil with $Cr^{+++}$, $Mn^{++}$ or $Pb^{++}$. In the presence of $Ni^{++}$ the necrosis appeared rapidly and frequently preceded marked chlorosis. The severity of chlorosis was judged to be in the order :—

$$Co^{++} > Cu^{++} > Zn^{++} = CrO_4'' = —Fe > Ni^{++} > Cr^{+++} > Mn^{++} > Pb^{++}$$

The order was similar in the citrate and magnetite series although certain differences were noted. Thus, in the citrate series the chlorosis caused by $CrO_4''$ or $Mn^{++}$ and the effects of different concentrations were more marked. The effects of $Cu^{++}$, $Zn^{++}$ or $CrO_4''$ respectively were comparable in the two series but $Co^{++}$ and $Ni^{++}$ produced a more severe injury in the magnetite series. Evidence was obtained, by painting the leaf surface with 0·25% $FeSO_4 7H_2O$ solution and a wetting agent, that the chlorosis was *in all cases* due to failure in the iron metabolism. A response was evident in about 48 hours and complete recovery of the treated area occurred in five days, while untreated leaves remained chlorotic. Plants given $Ni^{++}$ were too badly injured for treatment. Plants given $Cr^{+++}$, $Mn^{++}$ or $Pb^{++}$ grew with normal vigour ; the others were dwarfed. Fading, tinting or other types of necrosis

TABLE I.

Approximate Iron/Manganese Ratios in Nutrient Solutions and Yields of Fresh Material.

| | Experiment with Oat | | | | | | Experiment with Sugar Beet | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Fe p.p.m. | Fe $^{+++}$ m.e.q./L. | Mn p.p.m. | Mn $^{++}$ m.e.q./L. | Fe $^{+++}$/Mn $^{+++}$ ratio of equiv-alents | Yield in gms. 20 plants | Fe p.p.m. | Fe $^{+++}$ m.e.q./L. | Mn p.p.m. | Mn $^{++}$ m.e.q./L. | Fe $^{+++}$/Mn $^{++}$ ratio of equiv-alents | Yield in gms. 12 plants | Visual estimate of iron deficiency* |
| $Fe_0$ $Mn_0$ | ·005 | ·0003 | ·005 | ·0002 | 1·5 | 63·3 | ·005 | ·0003 | ·005 | ·0002 | 1·5 | 795 | NIL |
| $Fe_1$ ,, | 1·125 | ·06 | ·006 | ·0002 | 300 | 73·8 | 1·125 | ·06 | ·006 | ·0002 | 300 | 536 | NIL |
| $Fe_2$ ,, | 5·6 | ·3 | ·010 | ·0004 | 750 | 97·4 | 5·6 | ·3 | ·010 | ·0004 | 750 | 559 | NIL |
| $Fe_3$ ,, | 22·4 | 1·2 | ·027 | ·001 | 1200 | 65·3 | 28·6 | 1·5 | ·033 | ·0012 | 1250 | 1053 | NIL |
| $Fe_0$ $Mn_1$ | ·005 | ·0003 | ·555 | ·020 | 0·015 | 31·3 | ·005 | ·0003 | ·280 | ·01 | 0·03 | 1672 | 12 + + |
| $Fe_1$ ,, | 1·125 | ·06 | ·556 | ·020 | 3·0 | 338·9 | 1·125 | ·06 | ·281 | ·01 | 6·0 | 2575 | 4 + |
| $Fe_2$ ,, | 5·6 | ·3 | ·560 | ·020 | 15·0 | 360·3 | 5·6 | ·3 | ·285 | ·01 | 30·0 | 2339 | 1 + |
| $Fe_3$ ,, | 22·4 | 1·2 | ·577 | ·021 | 57·2 | 341·7 | 28·6 | 1·5 | ·303 | ·011 | 136·5 | 2462 | NIL |
| $Fe_0$ $Mn_2$ | ·005 | ·0003 | 5·50 | 0·2 | 0·0015 | 98·6 | ·005 | ·0003 | 2·75 | ·10 | 0·003 | 1491 | 12 + + + |
| $Fe_1$ ,, | 1·125 | ·06 | 5·50 | 0·2 | 0·3 | 336·5 | 1·125 | ·06 | 2·75 | ·10 | 0·6 | 2653 | 6 + + |
| $Fe_2$ ,, | 5·6 | ·3 | 5·51 | 0·2 | 1·5 | 370·9 | 5·6 | ·3 | 2·76 | ·10 | 3·0 | 2146 | 3 |
| $Fe_3$ ,, | 22·4 | 1·2 | 5·53 | 0·2 | 6·0 | 343·1 | 28·6 | 1·5 | 2·78 | ·10 | 15·0 | 2177 | 1 |
| $Fe_0$ $Mn_3$ | ·005 | ·0003 | 16·50 | 0·6 | 0·0005 | 112·4 | ·005 | ·0003 | 27·5 | 1·0 | 0·0003 | 1135 | 12 + + + + |
| $Fe_1$ ,, | 1·125 | ·06 | 16·50 | 0·6 | 0·1 | 336·9 | 1·125 | ·06 | 27·5 | 1·0 | 0·06 | 2549 | 12 + + |
| $Fe_2$ ,, | 5·6 | ·3 | 16·51 | 0·6 | 0·5 | 377·2 | 5·6 | ·3 | 27·51 | 1·0 | 0·3 | 2783 | 5 + |
| $Fe_3$ ,, | 22·4 | 1·2 | 16·53 | 0·6 | 2·0 | 360·9 | 28·6 | 1·5 | 27·53 | 1·0 | 1·5 | 2633 | 1 |

* The number of + signs represents the severity of chlorosis based on appearance, and the numeral, the number of affected plants.

also developed in the older leaves. These symptoms, described in detail in the appendix, were all distinct from those associated with iron deficiency. The general order of toxicity, as distinct from chlorosis, was judged visually to be as follows :—

$$Ni^{++} > Co^{++} > Zn^{++} > Cu^{++} > CrO_4'' > Cr^{+++} = Mn^{++} \text{ and } Pb^{++}$$

The symptoms in some of the older leaves of plants given extra $Zn^{++}$ resembled those produced by manganese deficiency and occurred simultaneously with iron deficiency in the young leaves of the same plant. Determinations of soluble manganese (17) (appendix) indicated a low manganese status.

**1948 Experiment.**

(a) Sugar Beet.

The effects of $Cr^{+++}$, $Mn^{++}$, $Co^{++}$ etc., on sugar beet were similar to those observed in 1947. $CrO_4''$ appeared to be rather more active in inducing chlorosis than before and was at least equal to $Cu^{++}$ in this respect in 1948. $Cd^{++}$ was found to produce acute chlorosis as well as other effects sometimes seen also in potassium deficiency (see appendix). $VO_3'$ also induced marked chlorosis and $MoO_4''$ was active, but less so. $WO_4'$ did not induce any chlorosis. The relative ability of the metal ions to produce chlorosis in sugar beet is shown below. Each ion is given in the order of visual severity of chlorosis.

| | | |
|---|---|---|
| Severe chlorosis :— | | $Co^{++}$, $Cd^{++}$, $CrO_4''$, $Cu^{++}$ |
| Moderate | ,, | $Zn^{++}$, $VO_3'$, $Ni^{++}$, $Cr^{+++}$, $MoO_4''$ |
| Slight | ,, | $Ti^{+++}$, $Mn^{++}$, $Pb^{++}$, $AsO_4''$ |
| Trace | ,, or nil | $Sn^{++}$, $Ce^{++++}$, $WO_4'$, $Tl^{++}$ |

Extra $MoO_4''$ in addition to the other ions *greatly* accentuated chlorosis and depression of growth when given with $Mn^{++}$, $Cr^{+++}$, $Cu^{++}$ or $Zn^{++}$. The effect of extra $MoO_4''$ was particularly striking in the presence of $Mn^{++}$ as shown in Plate III, *Fig.* 2. The interaction with $CrO_4''$, $Co^{++}$ and $Ni^{++}$ was barely perceptible and there was possibly a slight alleviation of the effects caused by $Co^{++}$. The omission of iron in the presence of extra $MoO_4''$ resulted in more acute chlorosis and was followed by symptoms apparently of boron deficiency, seen also much later and less pronounced in the normal omitting iron treatment. This diagnosis was supported by tissue test (see appendix).

The plants grown with urea as a source of nitrogen were smaller and darker green than those in the basal treatment. When iron was omitted iron deficiency symptoms were less marked than in the basal treatment. When extra $Cu^{++}$ and $Zn^{++}$ were included, the symptoms of induced iron deficiency were delayed by 3—4 weeks for $Cu^{++}$ and 8—10 weeks for $Zn^{++}$, but the plants were ultimately *more* chlorotic and stunted than those in the corresponding nitrate series.

(b) Tomato, Potato, Oat, and Kale.

The relative ability of the metal ions to induce chlorosis varied greatly according to the crop, but the order of activity was usually similar to that shown for sugar beet, with certain exceptions. The symptoms produced, however, did not always resemble those of true iron deficiency.

$CrO_4''$ caused marked reduction in vigour in tomato and potato, but not in kale or oat ; slight chlorosis appeared in tomato. $Cr^{+++}$ was much

less toxic to growth but produced typical slight iron deficiency symptoms in tomato.

No chlorosis at all appeared in any of these plants given extra $Mn^{++}$. Slight and moderate symptoms of typical manganese toxicity were recorded in kale, tomato and potato.

$Co^{++}$ proved extremely toxic to oat (more so than any other treatment) and induced acute chlorosis typical of severe iron deficiency, and a characteristic leaf reflexing effect as shown in Plate III, *Fig.* 3. $Co^{++}$, however, induced unusual symptoms in tomato and potato, described in detail in the appendix. The outstanding visual effects appeared to be the *increase* in chlorophyll in veins, leaf margins and in lower leaves, which were dark blue-green ; younger leaves showed bright intervenal mottling with necrosis and leaf malformation in tomato, and two distinct patterns in potato, one resembling iron deficiency and one of a novel appearance (Plate III, *Fig.* 4). Kale exhibited a pattern distinct from that of iron deficiency, in addition to other effects. $Co^{++}$ was highly injurious to all crops grown.

$Ni^{++}$ also caused unexpected effects. Chlorosis was induced in potato and was accompanied by necrotic spotting in a pattern closely resembling manganese deficiency. The symptoms produced in tomato prior to rapid death of the shoot also resembled manganese deficiency but no symptoms resembling this disorder appeared in kale or oat. Kale developed severe leaf necrosis and oat showed a characteristic *oblique* banding of alternating green and white areas ; later tillers were totally yellow. $Ni^{++}$ was the most injurious of all the metal ions except for oat.

$Cu^{++}$ induced symptoms indistinguishable from acute iron deficiency in tomato and potato, but no chlorosis appeared in oat or kale and none of these crops were as severely injured as sugar beet.

$Zn^{++}$ depressed growth in kale and slightly in oat and tomato ; potato was more tolerant. Symptoms of iron deficiency were slight and only temporary in oat, kale and tomato.

The relative effects on growth are indicated by the data in Table II.

TABLE II.

Relative Yields as percentage of Yield in Basal Treatment.

| Treatment | Potato | | | Oat Straw and Grain | Kale Leaf and Stem | Tomato Leaf and Stem |
|---|---|---|---|---|---|---|
| | Tubers | Haulm | Total | | | |
| Omitting iron .. | 83 | 83 | 83 | 35 | 8 | 36 |
| Extra $CrO_4''$ .. | 61 | 49·5 | 58 | 62 | 87 | 15 |
| „ $Cr^{+++}$ .. | 81 | 92·5 | 84 | 90 | 97 | 86 |
| „ $Mn^{++}$ .. | 92 | 84 | 89 | 132 | 84 | 84 |
| „ $Ni^{++}$ .. | 22 | 3·5 | 18 | 48 | 9 | 7 |
| „ $Co^{++}$ .. | 49 | 13 | 40 | 1 | 23 | 34 |
| „ $Cu^{++}$ .. | 51 | 67 | 58 | 79 | 100 | 51 |
| „ $Zn^{++}$ .. | 80 | 64 | 76 | 76 | 64 | 93 |

## DISCUSSION.

The conclusions drawn by Shive (20), (21), and others (18), (23), on the inter-relationships of iron and manganese require examination in relation to the results obtained in these experiments ; other possible causes of metal-induced iron deficiency also merit consideration. In experiments by the workers referred to, more importance was attached to the ratio of manganese to iron in the nutrient solution than to their absolute concentrations. Shive and others stressed the *reciprocal* nature of the relationship between these two elements and concluded that effects of a deficiency of one were identical with those of an excess of the other.

These considerations were based on two assumptions. First, that metabolically active iron exists in plants in the ferrous state, although valid evidence for this assumption, which has been criticised by Arnon (1), is still lacking. Second, that excess of manganese inactivates the " active " iron by oxidation to the ferric state, when it is precipitated as an " organic phosphorus " compound. This oxidation was ascribed to the fact that trivalent manganese has a higher redox potential with respect to the divalent ion than that of the ferrous-ferric potential. A similar effect was forecast for cobalt, where the valency change is associated with a still higher potential, and evidence was obtained that cobalt was in fact more active than manganese.

The importance of the manganese-iron ratio in the appearance of deficiency symptoms of either iron or manganese is only partly borne out by the results of the experiments described here. Thus *simultaneous* symptoms of iron and manganese deficiencies appeared in the *same* leaves of oat plants given low levels of both elements. Simultaneous symptoms of iron and manganese deficiencies have been seen also in oat in two independent centres in the field in separate years.* Specific morphological effects due to the simultaneous deficiency of these elements have also been recorded in *Theobroma cacao* (4). Furthermore the growth of oat and sugar beet was limited chiefly by the absolute levels rather than the ratio of iron and manganese supplied. When manganese was deficient growth was increased by increase in the iron supply, presumably on account of the trace of manganese carried by the ferric citrate, in spite of the fact that the Fe/Mn ratio was actually greater. The general incidence of chlorosis in sugar beet was clearly affected by the *level* of iron as well as by the iron-manganese ratio. The severity was greater in the $Fe_1$ $Mn_2$ set than in the $Fe_2$ $Mn_3$ set although the latter had a greater Mn/Fe ratio. Observations made on several crop plants between 1944 and 1948 (6), (7), (8), (10), (25), have shown that excess of manganese induces characteristic symptoms different from those of iron deficiency. These symptoms may be produced without accompanying chlorosis, which even when present seldom resembles iron deficiency.

The visual results of these experiments confirm that several metal ions in addition to manganese may induce symptoms of iron deficiency in plants and many of the metals studied are far more active than manganese. Crop susceptibility also varied considerably. This was particularly so for $Cu^{++}$, which induced chlorosis in sugar beet, potato and tomato but not in oat or kale—two plants susceptible to lack of iron. A similar distinction applied to $CrO_4''$. In several instances the chlorosis produced was distinct from the true symptoms of iron deficiency. The apparent *intensification* of chlorophyll

* Observations of T. Wallace, E. J. Hewitt, D. J. D. Nicholas on effects of liming acid soil after application of metal containing sewage residues, and of W. Plant on effects of spreading calcareous sand on soil in the vicinity of mine dumps.

content in leaf margins and veins and in older leaves of tomato and potato caused by $Co^{++}$ was particularly striking; in oat and sugar beet on the other hand the effects appeared to be those of acute iron deficiency.

$Ni^{++}$ also caused unusual effects. The simulation of manganese deficiency in potato and tomato and the effects of $Zn^{++}$ on sugar beet in 1947 suggest that metal deficiencies other than of iron may also be induced by metal excesses. The striking effects of $Ni^{++}$ in oat were unlike any previously recorded except when oat plants have been injured by simultaneously high concentrations of nickel and cobalt in an acid soil*. The symptoms produced in late tillers, however, resembled severe iron deficiency. The effects of $Ni^{++}$ would appear to be complex.

The suggestion (20) that relative oxidation-reduction potentials are responsible for the chlorosis induced by manganese or cobalt requires examination on the basis of the results obtained here. It is clear that manganese and cobalt are in no way unique in their capacity to induce chlorosis. Of the others found to possess this capacity at least one, namely zinc, does not undergo the valency change associated with simple oxidation-reduction reactions. The metal ions examined were found to differ considerably in their activity according to the crop concerned and it is evident that results obtained with a single crop, e.g. soya bean, should not be used as the basis for generalisation. Of the metal ions used $Mn^{++}$ was one of the least active and $Ce^{++++}$ was totally inactive in inducing iron deficiency, whilst $Cu^{++}$, $CrO_4''$, $Cd^{++}$ and $Co^{++}$ were often the most active, although the effects of the latter were not always typical of iron deficiency. The oxidation-reduction potentials of the simple ions as given by Latimer (14), however, show no relation to these results. Thus the $E_0$ values for the oxidation reduction changes involved in $Ce^{+++} \rightarrow Ce^{++++}$; $Mn^{++} \rightarrow Mn^{+++}$; $Co^{++} \rightarrow Co^{+++}$; $Cu^{+} \rightarrow Cu^{++}$; $Cr^{++} \rightarrow Cr^{+++}$, and $CrO_4'' \rightarrow Cr^{+++}$ are as follows:— —1·61; —1·51; —1·84; —0·167; +0·41, and —1·36. The $E_0$ value for $Fe^{++} \rightarrow Fe^{+++}$ is —0·77. On the simple hypothesis of Shive and others at least three, namely $Cu^{++}$, $Cr^{+++}$ and also $Cd^{++}$, should be incapable of oxidising the postulated "active" ferrous iron. This hypothesis also means that as manganese and cobalt were given in the divalent form they must first be oxidised to the higher valency state by reaction in a system that, in their absence, does not disturb the normal ferrous-ferric equilibrum. Further, the existence in living tissues of the free trivalent cobalt ion would be unlikely as the potential is sufficient to decompose water.

On the other hand many of the metals studied would probably be combined as complexes after absorption by the plant. The oxidation-reduction potentials of *complex* ions may, however, differ greatly from, and bear no relation to, those of *simple* ions, and thus the potentials produced relative to iron also in complex form would probably be very different. Manganese or cobalt for example in *complex* form would exist more readily in the trivalent than in the divalent state. Subsequent decomposition of such complexes would produce momentarily a powerful oxidising potential which, if coupled to a system involving iron, might result in maintaining this element in the postulated inactive (oxidised) state. Such a mechanism would not, however, account for the effects produced by copper, cadmium, or zinc. Therefore any explanation of the observed effects in terms of oxidation potentials must await

* These effects were recognised by Dr. R. L. Mitchell of the Macaulay Institute for Soil Research as resembling those seen in oat showing injury in an acid soil containing an excess of nickel and cobalt together.

more detailed knowledge of these metal compounds and of the precise nature of "active" iron in plants. Hypotheses based on oxidation-reduction potentials of *simple* ions are inadequate to explain the facts observed here.

Other possibilities which may be responsible for the production of symptoms of iron deficiency must be considered. It is unlikely that chlorophyll production is the only reaction involving iron, as this element is known to be required in catalase and cytochrome enzyme systems. At present, however, it is not possible to distinguish *visually* between effects caused either by interference in the specific function of iron in chlorophyll formation or by competition between this and other reactions for limited amounts of available iron, if the iron requirement in one of these other reactions is increased by the presence of an excess of one of the toxic metals. Thus the localisation of the copper-containing polyphenol oxidase system in the chloroplasts of spinach beet (2), may be of particular interest in this connection. It is possible that some of the metals studied may compete with iron simply by blocking a reaction leading to chlorophyll synthesis owing to similarity in their ionic radii, valency and electron configurations. Bennett (3) and Jacobsen (13) have suggested that "active" iron may be closely associated with a particular protein fraction in leaves and some of the metals considered may cause inactivation by precipitation or denaturation of these proteins or by adsorption on them with consequent interference with their function. The unusual apparent distribution of chlorophyll in some plants given extra cobalt may imply that the distribution of "active" iron has been modified and close observation of *patterns* in the chlorosis should assist in the study of this possibility, which may also apply to localisation of iron in the roots at the expense of the leaves, for which there is some evidence.

The interactions of molybdenum with some of the metals given to sugar beet (in sand culture) were contrary to those reported by Millikan (15), (16), for flax in water culture. In either case, however, the effect of molybdenum suggests that some aspect of nitrogen metabolism is involved since molybdenum is required in some stage of nitrate reduction. Increased molybdenum supply might increase the rate of this and related reactions and thus interfere with the ferrous-ferric equilibrium or increase the demand for available iron. The inclusion of urea as a source of nitrogen was intended as a check on the interpretation of the effect of molybdenum but the results were inconclusive. Unpublished observations obtained from other experiments on the relation of iron deficiency to potassium supply in potato* also show that chlorosis may be reduced when urea is used in place of nitrate nitrogen and it is a common occurrence for iron requirements to be reduced when ammonium salts are used.

## CONCLUSIONS.

The evidence considered here shows that the problems of metal-induced iron deficiency are complex. Manganese is not unique in its ability to cause chlorosis and several metals are far more active in this respect. Observations of simultaneous symptoms of iron and manganese deficiencies in the same plant, and the distinction between manganese toxicity and iron deficiency, clearly imply some independent functions for these elements.

The functional status of ferrous and ferric iron in chlorophyll formation is still not clear and the cause of induced iron deficiency cannot yet be specified in terms of their equilibrium. Hypotheses based on the relative oxidation-

* E. W. Jones, Unpublished Data.

reduction potentials of *simple* ions of the metals considered appear inadequate to explain their activity and more than one mechanism may be involved.

## SUMMARY.

1. This paper describes sand culture experiments on sugar beet, oat, tomato, potato and kale in which the effects of excess of manganese, chromium, cobalt, nickel, copper, zinc and other metals on the iron status of these crops were studied.

2. Experiments on the effects of varied iron-manganese ratios in oat and sugar beet *did not* confirm the hypothesis that excess of one and deficiency of the other are synonymous. There is considerable evidence that these elements have independent functions.

3. Several metals, particularly cobalt, copper, cadmium and chromium as chromate, induced symptoms of iron deficiency in sugar beet; manganese was one of the least active in this respect.

4. The chlorosis in potato and tomato caused by copper was always typical of iron deficiency but symptoms produced by nickel and cobalt in these crops and in kale and oat were either unlike true iron deficiency or were accompanied by other unusual features.

5. The crops varied in their relative sensitivity to the several metal ions studied.

6. Additional molybdenum greatly accentuated the chlorosis in sugar beet produced by chromium as chromic sulphate, manganese, copper and zinc.

7. The possible mechanisms involved in the production of induced iron deficiency symptoms are examined. Explanations based on oxidation-reduction potentials of *simple* ions are considered inadequate and alternative possibilities are considered.

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## APPENDIX.

## Description of Visual Symptoms.

### 1946 Experiment.

### Oat.

*Omitting Iron.* Growth severely stunted, tillering restricted; younger leaves paling from base, with intervenal chlorosis extending to leaf tip; successive leaves more chlorotic, finally totally pale yellow, with bleached lesions and withering from tips; failure of flower and premature death.

*Omitting Manganese.* Growth severely stunted, tillering restricted; foliage dull and lustreless, often limp and "damp" to feel; colour pale with slight intervenal chlorosis except $Fe_3$ $Mn_0$ treatment; intervenal grey speck lesions with grey or blue-green border commencing as dull grey-green watersoaked area and drying out to pale brown in centre; older leaves, first lesions close to cleaf base and appearing nearer to apex in successive leaves; lesions spreading longitudinally and fusing laterally to cause backward collapse of leaves; leaves finally mainly withered except extreme tip; flowering and ear formation prevented, premature death.

*Omitting Iron and Manganese.* Growth and tillering as above; foliage chlorotic, intervenally yellow or white, some youngest leaves totally yellow; typical grey speck lesions in leaves with intervenal chlorosis mainly in lower regions, lesions coalescing to cause collapse of leaf; flowering prevented.

### Sugar Beet.

*Omitting Iron.* $Fe_0$ $Mn_1$:—Growth stunted; foliage chlorotic, intervenal areas yellow green or ivory colour, all veins clearly visible pale green producing a "fine pattern"; youngest leaves often pale green.

$Fe_0$ $Mn_2$:—Growth stunted; similar pattern but still more clearly defined, and in some leaves major veins only remaining pale green, with large pale brown intervenal necrotic areas.

$Fe_0$ $Mn_3$:—Growth severely stunted; chlorosis more severe, middle leaves often totally yellow or only midrib and lateral main veins pale green, younger leaves totally chlorotic; larger glistening brown necrotic areas covering most of leaf except tip and larger veins.

*Omitting Manganese.* Growth stunted; erect habit and leaves brittle, stellate or triangular outline due to incurling of margins; foliage pale green with diffuse intervenal mottling obscuring minor veins and producing a "coarse pattern," major veins often indistinct; intervenal areas yellow-green or dull yellow with irregular scattered necrotic areas usually sunken in undersides and finally perforating. Pattern more diffuse in $Fe_1$ and $Fe_2$ treatments; $Fe_3$ $Mn_0$ darker green.

*Omitting Iron and Manganese.* Growth as above; typical symptoms of manganese deficiency as produced in $Fe_1$ $Mn_0$ treatment.

### 1947 Experiment.

### Sugar Beet.

*Omitting Iron.* Growth stunted, "fine pattern" intervenal mottling in younger leaves when moderate, becoming total yellow chlorosis when severe; irregular brown necrotic areas between major veins, older leaves remaining green or fading slightly prior to death of plant.

*Extra $CrO_4''$.* Growth stunted, leaf symptoms generally typical of severe iron deficiency, major veins only pale green or leaves totally chlorotic; necrotic areas more extensive than in simple iron deficiency; marked pink tinge in petiole of younger leaves.

*Extra Cr* + + +. Growth normal or slightly reduced, foliage erect; pronounced fine pattern mottling of middle and younger leaves, with yellow or ivory intervenal chlorosis as in iron deficiency; occasional small irregular necrotic areas; effects more pronounced in citrate series.

*Extra Mn* + +. Resembling Cr + + but still less effect on growth; chlorosis typical of slight iron deficiency; few necrotic areas; generally more marked in citrate sets.

*Extra Ni* + +. Growth severely stunted, particularly in magnetite series; foliage narrow, leaf margins inrolled; rapid appearance of yellow-green or yellow chlorosis of middle and younger leaves but youngest leaves sometimes greener, chlorosis generally continuous rather than mottled; severe intervenal necrosis of middle and young leaves often preceding marked chlorosis and beginning as narrow brown areas adjacent to major veins, spreading to whole intervenal area, dark brown or black especially in young leaves, finally involving whole leaf; loss of lamina and death of youngest leaves and of plant, especially in magnetite series; old leaves faded with yellow or bright orange tinge between veins; roots brown, stunted and undeveloped.

*Extra Co* + +. Growth practically as for Ni + +; foliage narrow, with inrolled margins; severe and rapid development of yellow intervenal chlorosis in middle and youngest leaves rapidly becoming total chlorosis and soon followed by necrosis; green veins more evident than in Ni + +, necrosis appearing as for Ni + + but more central in leaf, more regular and major veins remaining clearly defined pale green for longer period after total blackening of intervenal tissue; finally youngest leaves blackened and nearly devoid of lamina; middle leaves bronzed tint before showing necrosis; old leaves grey-green, later bronze mottling with wilting and scorching; roots stunted and brown; death of plants especially in magnetite series.

*Extra Cu* + +. Growth severely stunted, but slightly better than with Co + + or Ni + + and unaffected by source of iron; young leaves acute bright yellow intervenal (minor veins not distinguished), or total yellow chlorosis, with considerable irregular intervenal brown necrotic areas of middle leaves; pink tinge of young petioles; old leaves dull green; death of plants occurred gradually.

*Extra Zn* + +. Growth resembling extra Cu + + plants but habit somewhat rosette and "open"; fine pattern dull yellow chlorosis of younger leaves, major veins and often some minor veins usually clearly defined, total chlorosis infrequent; irregular necrotic intervenal areas finally involving greater part of youngest leaves; middle leaves pale green, sometimes mottled; older leaves upcurled margins, diffuse pale orange or yellowish intervenal mottling, major veins dark green and diffusely outlined, in some leaves similar to effects of manganese deficiency in pattern and in appearance of occasional speckled necrotic areas; later becoming bleached to dull yellow tinge; death of some plants. Tissue test value for Basal Mn: 0·05 p.p.m., Zn at 0·5 m.e.q./L.:—0·015 p.p.m., Zn at 1·0 m.e.q./L.:—0·005 p.p.m.

*Extra Pb* + +. Growth normal in citrate set, ultimately slightly squat in magnetite set, habit erect; slight intervenal yellow-green mottling of middle or younger leaves, either clearly defined pattern or somewhat diffuse, necrosis almost nil.

## 1948 Experiment.

### Sugar Beet.

*Omitting Iron.* Typical symptoms of iron deficiency varying from pale foliage with fine pattern chlorotic mottling between veins to total chlorosis of younger leaves. Growing points normal on Aug. 23rd. By Oct. 6th one plant showed death of growing point, one blackening of young leaves and two "cross hatching" of petioles (c.f. notes in next paragraph).

*Omitting Iron with Extra $MoO_4''$.* Symptoms of iron deficiency greatly accentuated, mainly severe, total chlorosis of younger leaves with necrotic areas; youngest leaves becoming dwarfed and narrow, blackening at leaf base followed by death of growing point, and "cross hatching" on petioles of middle leaves; symptoms apparently identical with boron deficiency (diagnosis supported by tissue test carried out 23.8.48 for boron (L) compared with omitting iron only (M)*, whilst adequate and equal amounts of boron were found to be present in the respective nutrient solutions). 5 out of 9 plants affected by death of growing point on Aug. 23rd. By Oct. 6th three plants were dead, three showed dead growing point, two showed "cross hatching." The older leaves were thickened and orange tinted.

*Omitting Iron (Nitrogen as Urea).* Growth reduced, foliage mainly green, or slightly mottled; only two plants moderate chlorosis effects.

*Basal treatment (Nitrogen as Nitrate, Iron as Ferric Citrate).* Growth normal, slight chlorosis in early stages owing to temporary omission of iron, followed by recovery and normal foliage.

*Basal treatment with extra $MoO_4''$.* Growth normal; foliage showing typical and persistent slight to moderate iron deficiency symptoms.

*Nitrogen as Urea, Iron as Ferric Citrate.* Growth reduced; leaves small, dark green.

*Basal treatment with extra $CrO_4''$.* Growth dwarfed, typical severe iron deficiency, younger leaves mainly totally chlorotic; plants killed-out by end of September.

* The tests were made by Dr. D. J. D. Nicholas whose assistance is gratefully acknowledged.

*Basal treatment with extra $CrO_4''$ and $MoO_4''$.* Similar to above, chlorosis similar or still more marked in some plants, no difference in apparent degree of toxicity.

*Basal treatment with extra Cr + + +.* Growth slightly reduced ; slight typical iron deficiency chlorosis.

*Basal treatment with extra Cr + + + and $MoO_4''$.* Growth considerably reduced ; moderate to severe chlorosis, typical pattern of iron deficiency, plants killed.

*Basal treatment with extra Mn + +.* Growth normal chlorotic effects trace or nil.

*Basal treatment with extra Mn + + and $MoO_4''$.* Growth stunted ; chlorosis symptoms typical or moderate to severe iron deficiency, including complete chlorosis of younger leaves and necrosis.

*Basal treatment with extra Ni + +.* Growth severely dwarfed, followed by rapid death ; pale yellow green chlorosis of younger leaves rapidly followed by black necrosis between major veins, death of youngest leaves and growing point ; orange mottling of older leaves ; blackening lateral roots and upcurling of top root.

*Basal treatment with extra Ni + + and $MoO_4''$.* As above, no apparent difference.

*Basal treatment with extra Co + +.* Growth severely dwarfed, followed by rapid death ; young leaves acutely chlorotic yellow, with dark brown intervenal necrosis, major veins pale yellow, rapidly becoming totally necrotic ; older leaves dull olive green with orange tint, inrolled margins ; roots brown and coarse, top root upcurled.

*Basal treatment with Extra Co + + and $MoO_4''$.* Similar but death of plants slightly delayed ; early symptoms of chlorosis slightly less severe.

*Basal treatment with extra Cu + +.* Growth stunted followed by death ; severe chlorosis and necrosis.

*Basal treatment with extra Cu + + and $MoO_4''$.* Similar, but more chlorotic and killed more rapidly.

*Nitrogen as Urea with extra Cu + +.* Growth dwarfed ; foliage at first greener than corresponding nitrate set developing fine pattern chlorotic mottling with dark veins and finally chlorosis much more acute, leaves totally yellow, older leaves limp later withered.

*Basal treatment with extra Zn + +.* Growth reduced ; moderate chlorotic effects typical of iron deficiency ; one plant showing diffuse intervenal fading and dark green veins suggestive of manganese deficiency pattern.

*Basal treatment with extra Zn + + and Mo.* Growth reduced ; moderate to severe iron deficiency followed by premature death of some plants. Symptoms suggestive of manganese deficiency in two plants at first but later disappearing.

*Nitrogen as Urea with extra Zn + +.* Growth dwarfed ; leaves small, dark green at first, chlorosis delayed ; later fine net veined pattern with clearly defined dark green veins ; older leaves becoming limp and faded, finally withering.

*Basal treatment with extra Ti + +.* Growth slightly reduced ; foliage dull green ; later slight mottling.

*Basal treatment with extra $VO_3'$.* Growth reduced ; moderate to severe chlorosis resembling iron deficiency ; several plants killed.

*Basal treatment with extra $AsO_4''$.* Growth slightly reduced. Older leaves full, faded green with yellowish margins ; younger leaves pale, somewhat chlorotic between bright green veins.

*Basal treatment with extra Cd + +.* Growth markedly reduced ; intervenal chlorosis typical of iron deficiency rapidly becoming acute and extremely severe, total yellowing of all younger and middle leaves ; older leaves becoming completely bleached between pale green veins ; some plants showed dark brown watersoaked markings on the petioles of older leaves as in potassium deficiency, occasionally dark markings in main veins on undersides of leaves. These plants had a normal potassium status (by tissue test method) ; plants killed.

*Basal treatment with extra Sn + +.* Growth normal ; foliage green, older leaves somewhat faded.

*Basal treatment with extra Ce + + + +.* Growth normal ; foliage dark green, old leaves temporarily slightly dull and faded.

*Basal treatment with extra $WO_4'$.* Growth normal ; foliage dark green, older leaves slightly smaller than normal, rounded and slightly faded.

*Basal treatment with extra Tl + +.* Growth severely reduced ; foliage chlorotic with additional orange tinted blotching, intervenal crinkling and marginal scorching ; plants rapidly killed.

*Basal treatment with extra Pb + +.* Growth slightly reduced ; foliage dull green, slight chlorotic mottling, older leaves faded green. General Note : A positive response shown as chlorophyll formation was obtained after painting a small area of chlorotic leaf of sugar beet plants with 0·25% $FeSO_4\ 7H_2O$ and ester salts solution in all treatments showing chlorosis.

## Potato.

*Omitting Iron.* Growth slightly reduced ; foliage pale green, younger leaves yellow-green ; shoots yellowish but *no marked pattern between veins.*

*Basal treatment.* Normal.

*Basal treatment with extra $CrO_4''$.* Growth reduced ; foliage dark green, old leaves later faded yellow-green ; irregular crumpled dark brown necrotic areas at tips of margins of mid-stem, leaves visible both surfaces and spreading along midrib, leaves withering and leaf-drop ; occasional black necrotic markings on stems. (Pathogens apparently absent).

*Basal treatment with extra Cr* + + +. Growth slightly reduced, foliage pale, shoots slightly chlorotic as in *omitting iron* treatment.

*Basal treatment with extra Mn* + +. Growth slightly reduced ; typical symptoms of manganese toxicity as described elsewhere (7). No symptoms of chlorosis.

*Basal treatment with extra Ni* + +. Growth greatly reduced and stunted ; foliage pale, upper leaves showing fine pattern yellow-green intervenal chlorosis, small brown necrotic spots, at first angular later rounded, in regular pattern along midrib and major veins commencing in basal region of leaflets and resembling manganese deficiency ; older leaves suddenly fading and showing bright orange tint before withering and defoliation, petioles not speckled but elongated black necrotic areas in epidermis of petiole and stem ; death of growing point ; death and dropping of unopened flower buds ; tubers small, with deep elongated corky splits with blackened margins mainly at growing point end of tuber ; early death of plants.

*Basal treatment with Co* + +. Growth reduced ; foliage initially dark green, especially older leaves ; younger leaves paling, fine patterned bright yellow- or orange-green intervenal chlorotic pattern, veins dark green, leaf tip and margins also dark green ; midrib and petiole purple tint ; pattern differentiated into two types (a) as above but veins paler green, (b) major veins and irregular adjacent areas of mesophyll and leaf margins particularly dark green, irregular intervening " islands " of ivory coloured chlorotic tissue, pattern independent of minor veins and situated mainly in centre of leaflet, green areas plainly *darker* than in leaflets of normal plants ; dehiscence of flowers frequently before opening ; stems and petioles without any lesions ; tubers with corky elongated splits arranged in ring around growing point end ; death of plants.

*Basal treatment with extra Cu* + +. Growth slightly reduced ; foliage becoming pale, younger leaves pronounced pale yellow chlorosis identical with that produced by iron deficiency ; no distinctive pattern, major and minor veins faintly greener than intervenal areas, pale brown necrosis along midrib ; profuse pale brown speckling of pale green stems ; tubers normal.

*Basal treatment with extra Zn* + +. Growth slightly reduced ; foliage and tubers normal.

## Tomato.

*Omitting Iron.* Growth reduced ; foliage pale, younger leaves pale yellow-green, ivory tinted intervenal chlorosis commencing basal area of leaflets ; major and minor veins *pale* green making fine pattern, veins ultimately nearly as chlorotic as intervenal areas, petioles pink or pale purplish tint ; fruit silvery or grey-green before ripening.

*Basal treatment.* Growth and foliage normal.

*Basal treatment with extra* $CrO_4''$. Growth thin and dwarfed ; leaves small, dull faded green, faint yellowish intervenal mottling in younger leaves, veins normal green ; older leaves dull yellow tint and yellowing of margins, leaflets curling, withering and dropping ; few small fruit set.

*Basal treatment with extra Cr* + + +. Growth slightly reduced ; older leaves normal green, younger leaves typical symptoms of slight iron deficiency chlorosis, recovering later.

*Basal treatment with extra Mn* + +. Growth slightly reduced ; typical symptoms of moderate manganese toxicity as described elsewhere (7).

*Basal treatment with extra Ni* + +. Growth severely stunted ; rapid appearance of chlorotic orange or yellow-green mottling between bright green veins in upper leaves (symptoms resembling manganese deficiency), but more marked in basal regions of leaves, followed quickly by brown necrotic intervenal speckling ; leaflets folding up, necrotic symptoms spreading down to mid-stem leaves ; youngest leaves rapidly shrivelling, older leaves remaining dark green with purple tint and forward rolling of margins ; extensive brown scaly necrotic lesions and areas on petioles near leaf bases and stem below ; shoot near growing point brittle and followed by splitting ; death of growing point ; first flowers abnormally pale yellow, later trusses flowers aborted ; death of plants.

Tissue test data for Mn : Basal ·03 p.p.m., Ni + + ·002 p.p.m., Zn + + ·002 p.p.m.

*Basal treatment with extra Co* + +. Growth reduced ; younger leaves bright yellow-green intervenal mottling, fine pattern between dark green veins, leaf margins and clearly defined veins becoming exceptionally dark green or blue-green with purple tints, intervenal areas becoming bright yellow, with brown necrotic spotting close to major veins and midrib, later perforated and slit-like ; leaflets narrow, wavy, margins folded up, hooked at tips, finally distorted, rudimentary and lacking true lamina ; petiole curled with only peg-like projections ; shoot chlorotic, purple tint in midrib, petioles and shoot ; older leaves exceptionally dark blue-green ; death of growing point ; brown scaly lesions on stems, especially upper regions and often associated with leaf bases, internodes shortened ; first flowering trusses normal ; fruit small, developing ring of short longitudinal corky lesions around blossom end (c.f. potato tubers), later flower trusses extremely numerous, abnormal, flowers failing to set fruit, or remaining unopened or rudimentary ; roots coarse and non fibrous ; death of some plants.

*Basal treatment with extra Cu* + +. Growth reduced ; typical symptoms of iron deficiency ranging from initial paling of younger leaves with fine pattern intervenal yellow-green mottling commencing centre and basal area of leaflets, to bright ivory chlorosis between pale green veins and finally total pale yellow or ivory chlorosis and intervenal necrosis and withering of young leaves ; purple tinting in older leaves ; fruit on younger trusses silvery or grey-green before ripening.

*Basal treatment with extra Zn* + +. Growth slightly reduced ; one plant slight mottling of younger leaves as in mild iron deficiency.

## Kale.

*Omitting Iron.* Growth greatly reduced ; fine pattern yellow-green intervenal chlorotic mottling between pale green veins including minor veins, later becoming acute pale yellow or white chlorosis of whole leaf except for yellow-green tint in basal regions of veins, finally buff scorch and withering of leaf.

*Basal treatment.* Growth normal.

*Basal treatment with extra* $CrO_4''$. Growth slightly reduced ; no symptoms.

*Basal treatment with extra* $Cr^{+++}$. Growth normal ; no symptoms.

*Basal treatment with extra* $Mn^{++}$. Growth slightly reduced ; typical symptoms of slight manganese toxicity (8) i.e. leaf marginal cupping and necrotic spotting.

*Basal treatment with extra* $Ni^{++}$. Growth greatly reduced and stunted ; rapid appearance of fine pattern yellow-green intervenal mottling at first suggestive of iron deficiency, rapidly followed by profuse necrosis in extensive areas between veins, pale green areas immediately adjacent to veins, ultimately total intervenal scorch ; some leaves necrotic lesions in veins and on petioles ; younger leaves forward or backward tip hooking as in calcium deficiency followed by browning and death of whole lamina, ultimately rudimentary leaves with only bare stump-like petioles brown at tip ; death of growing point ; numerous axillary distorted shoots developed ; older petioles and stems hard scaly brown surface, green beneath ; roots brown and stunted ; death of plants.

*Basal treatment with extra* $Co^{++}$. Growth greatly reduced and stunted ; foliage either pale green rapidly developing acute yellow-green, later yellow, chlorosis between relatively dark green major veins followed by bleaching and withering (tint of chlorotic areas tended to *vary* between yellow-green to pale yellow and did not become progressively paler in all plants) ; or fine mottling mainly in marginal regions and profuse intervenal necrotic speckling ; narrow brown necrotic area around margins, marked forward marginal cupping (as in manganese toxicity) ; younger leaves sometimes completely " burnt " leaving only dried brown shreds of tissue hanging between veins, or totally yellow with brown margins and almost rudimentary ; older leaves finally total intervenal necrosis ; stems bronze or purple tint ; pith brown ; roots brown and rotted.

*Basal treatment with extra* $Cu^{++}$. Growth normal ; foliage with faint purple lustrous sheen.

*Basal treatment with extra* $Zn^{++}$. Growth reduced ; slight chlorosis of younger leaves later disappearing.

## Oat.

*Omitting Iron.* Growth greatly reduced ; typical symptoms of iron deficiency :—intervenal *longitudinal* yellow green striping later total chlorosis as described above.

*Basal treatment.* Growth normal.

*Basal treatment with extra* $CrO_4''$. Growth normal ; foliage green, trace of intervenal chlorosis in late tillers.

*Basal treatment with extra* $Cr^{+++}$. Growth slightly reduced ; no symptoms.

*Basal treatment with extra* $Mn^{++}$. Growth stimulated ; no symptoms.

*Basal treatment with extra* $Ni^{++}$. Growth reduced, foliage pale, unusual symptoms of pronounced irregular oblique banding of white chlorotic areas totally devoid of pigment alternating with normal green or slightly striped zones, commencing in mid-leaf region ; later younger foliage of late tillers also chlorotic but uniformly pale yellow, longitudinal intervenal chlorosis as in iron deficiency exceptional and only appearing late in season ; no symptoms suggestive of manganese deficiency.

*Basal treatment with extra* $Co^{++}$. Growth severely reduced and stunted, tillering suppressed ; acute intervenal yellow-green chlorosis of first leaf and total yellow chlorosis of succeeding leaves, leaf tip sometimes staying green ; brown necrotic lesions along leaf margins followed by bleaching and withering ; characteristic collapse of young leaves near base or little above producing sharply re-flexed angle ; early collapse and death of plants.

*Basal treatment with extra* $Cu^{++}$. Growth reduced ; no chlorosis, plants in one pot showed typical symptoms of slight manganese deficiency.

*Basal treatment with extra* $Zn^{++}$. Growth reduced ; foliage pale, slight intervenal chlorosis in late tillers.

*Fig.* 1. Left : Control$^{++}$ Fe as citrate.
Centre : Extra $Cu^{++}$ 0·5 m.eq. L.
Right : Extra $Mn^{++}$ „ „

*Fig.* 2. Left : Extra $Cr^{+++}$ 0·5 m.eq./L.
Right : Extra $Cr^{+++}$ and $MoO_4''$.

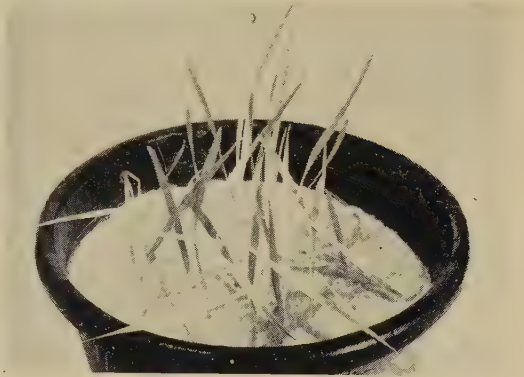

*Fig.* 3. Iron deficiency in oat caused by extra $Co^{++}$.

*Fig.* 4. Cobalt toxicity in potato. Two distinct patterns produced by excess of cobalt.
Left : Severe general chlorosis ; veins distinct.
Right : Blue-green areas adjacent to major veins and margins with excessive chlorophyll content ; internal, irregular, chlorotic areas.

*Fig.* 1. Red clover. Left : control. Remainder : molybdenum deficient : marginal paling, rolling and scorching.

*Fig.* 2. Kale : intervenal scorching, marginal inrolling and collapse of growing point.

*Fig.* 3. Swede seedlings. Left and centre : molybdenum deficient. Right : control.

*Fig.* 4. Swede, mature plants. Malformation of leaf lamina, tips browning and bare petioles.

# MOLYBDENUM AS A PLANT NUTRIENT.

## THE EFFECTS OF MOLYBDENUM DEFICIENCY ON SOME VEGETABLES, CEREALS AND FORAGE CROPS.

By E. J. HEWITT and E. W. JONES.

A previous paper (2) described a technique for nutrient and sand purification suitable to the study of molybdenum deficiency in plants and included observations of the symptoms produced by tomato and some Brassica crops. Certain symptoms produced by molybdenum-deficient cauliflower plants showed a striking resemblance to the field disorder long known as "Whiptail." This suggested the possibility that molybdenum deficiency in the field may affect the growth of other economic crops in addition to its recognised importance for leguminous forage crops (5). A survey is therefore in progress as part of a programme of work to observe the effects of molybdenum deficiency on the visual symptoms, yields and molybdenum content of a range of crop plants and to study the influence of molybdenum on their metabolism under varied conditions of nutrient supply. This paper gives some preliminary results obtained in 1948.

## EXPERIMENTAL.

### Materials and Methods.

The pyrex glass containers, sand, water and nutrient reagents used for this work were similar to those described in the first paper (2). Some modifications were made in their preparation, as follows :—

The sand was purified in batches, as before, but each batch was treated with three charges of 15% hydrochloric acid containing 1% of oxalic acid for three-hour periods, instead of two charges of acid for six-hour periods. Thus the average acid concentration was greater but the duration of the treatments was also shorter. The full details of these methods are in preparation for publication elsewhere (1).

The nutrient solutions were similar to those used previously but aluminium and gallium were included, each at 0·005 p.p.m. The stock solutions of major nutrients were also extracted after filtration (2) with redistilled chloroform and 8-hydroxyquinoline, and then with chloroform alone. One purpose of this modification was to remove traces of iron or other elements remaining in excess after precipitation by 8-hydroxyquinoline and finally to remove any excess of this reagent. The chloroform did not emulsify appreciably and good separation was obtained by leaving the solutions to stand for twelve hours or by a final extraction with redistilled ether.

The ferric citrate was prepared in a manner different from methods hitherto employed and the procedure described here is part of a more general method for freeing solutions from manganese, zinc, copper and gallium, as well as from molybdenum. 500 g. of twice-recrystallised ferrous ammonium sulphate were dissolved in the minimal amount of warm glass-distilled water, and about 0·75 g. of hydroxylamine were added. The solution was cooled, brought to a pH of 2·8 by the addition of concentrated hydrochloric acid, then extracted eight times with 0·1% solution of 8-hydroxyquinoline in chloroform and finally with chloroform alone. This extraction was intended

to remove gallium. It was followed by further extractions using sufficient analytical reagent grade hydrochloric acid saturated with redistilled ether to produce a final acid concentration of approximately 6N. after dilution with the ferrous sulphate solution. This extraction caused molybdenum and also gallium to pass into the ether phase. The concentration of ferrous iron present in solution was approximately determined with potassium permanganate and a stiochoimetric equivalent of hydrogen peroxide added to convert it to the ferric state. The peroxide had been purified, first by redistillation under reduced pressure in an all-glass apparatus and then by extraction at pH 2·8 with 8-hydroxyquinoline and chloroform until the latter appeared colourless. The ferric salt was concentrated on a water bath fitted with porcelain rings, recrystallised and redissolved in a minimal volume of warm glass-distilled water. It was then shaken vigorously with concentrated analytical reagent grade hydrochloric acid saturated with ether. The ethereal layer that separated was retained and the extraction repeated. The ethereal fractions were combined and washed with two successive portions of redistilled hydrochloric acid saturated with ether and the aqueous fractions were discarded. Finally the ether extract was shaken with three separate 200 ml. volumes of glass-distilled water which extracted all the ferric chloride, leaving the ether layer colourless. The combined aqueous extracts were concentrated to 100 ml. and extracted with 50 ml. portions of a freshly prepared 0·5% solution of dithiol in isoamyl acetate. The ferric iron concentration was determined. An equivalent amount of twice-recrystallised citric acid was added and the solution evaporated with frequent stirring in a silica dish to a powder. It was then dissolved in glass-distilled water and stored in a pyrex glass flask in the dark. One batch of ferric citrate was sufficient to meet the requirements of this experiment for the whole season. The stock ferric citrate solution was prepared by dilution of a suitable aliquot with glass-distilled water. Unpublished work, using *Aspergillus niger* as a test organism, showed that the ferric chloride so prepared was virtually free from molybdenum.

The crops and varieties grown were: Alsike clover, red clover (Dorset marl), lucerne (Grim strain), pea (Onward), dwarf bean (Masterpiece), broad bean (Long pod), barley (Plumage archer), rye, oat (Red Algerian), perrennial rye grass (Aberystwyth S.23), cocksfoot (Aberystwyth S.37), Yorkshire fog (*Holcus lanatus*) and Purple Molinia (*Molinia caerulea*), kale (Marrowstem), swede (Purple top), sugar beet, flax (Liral prince), celery (Giant white), tomato (Market king).

The barley, rye, kale and sugar beet seeds were germinated on glass wool in purified nutrient solution and then transplanted to their respective beakers on 20/5/48. On the same date the other crops were sown directly into the beakers. Germination was rapid, except in the case of Molinia where germination was extremely bad—one seedling appearing in the " molybdenum omitted " set and eight in the " molybdenum added " set. The cereals and grasses were thinned to ten plants per beaker, other plants were limited to 3-5 at first and later, in some instances, reduced to 1 or 2. Two or three beakers were allotted for each crop in the " molybdenum omitted " series whilst, owing to limited space, only one was allotted to each in the " molybdenum added " (i.e. control) series. In some instances cotyledons (of pea, bean) and endosperm (of cereals) were excised in both control and " molybdenum omitted " groups but no differential effects were noted and this will not be referred to again.

**Results.**

Characteristic visual symptoms for each molybdenum-deficient crop are given in an appendix. Some yield and chemical data are given in Tables I—IV.

*Visual observations.* The clovers and lucerne all showed a pale, dull grey-green tint in the young leaves followed by a severe white or buff coloured scorch and inrolling of the margins of the older leaves (*Plate IV Fig.* 1). No well defined symptoms were seen in pea or bean. The grasses showed few distinctive, easily recognisable foliage symptoms. The foliage of barley and rye was pale green and showed a lustreless grey tint, rye later became yellow-green. Oat showed a reddish brown tint and orange red lesions along the leaves which resembled possible drought effects. The Brassicae showed a typical diffuse intervenal yellow-green mottling followed by a wilting of grey-green marginal areas. The subsequent papery scorch of inrolled marginal regions was also characteristic. In kale the scorching also occurred in the intervenal areas, in one plant the growing point appeared on the verge of collapse (*Plate IV Fig.* 2) and divided into two late in the season after the onset of severe deficiency symptoms. Swede appeared particularly sensitive to molybdenum deficiency, and where severe the seedlings were killed 29 days from sowing after yellowing, wilting and withering of the expanding leaves (*Plate IV Fig.* 3). Late in the season swedes less severely affected showed also leaf malformations ; the younger leaves became narrow, distorted and cupped. They were finally brown and withered at the tips and lacking any lamina (*Plate IV Fig.* 4). Sugar beet showed incurled marginal leaf scorch when molybdenum was omitted and the foliage was yellow-green as with nitrogen deficiency ; celery also showed typical nitrogen deficiency symptoms, the foliage was pale and became practically yellow. Flax showed lower leaf scorch symptoms and wilting of the pedicels during flowering, a symptom associated with calcium deficiency and also seen in molybdenum deficient cauliflowers in 1946.

*Yields.* Replication was low and the yields given here (Table I) cannot be tested for significance. They are, however, indicative of the relative severity of the effects of molybdenum deficiency on growth. Growth reduction was greatest for the small seeded legumes (clovers and lucerne) and particularly so for alsike. Perennial rye grass seemed the most affected of the grasses. Seed yields were reduced in pea, broad bean and barley and these effects were more marked than those on growth as a whole. There was no consistent relationship between treatment and percentage dry matter in fresh weight.

*Chemical Data.* Fresh petioles of leaves were tested for nitrate content as in 1947 (Table II). The nitrate content of these tissues of molybdenum-deficient plants was consistently much greater than in control plants and the relative concentration often differed by a factor of ten or more. Root tissues were only tested twice and showed small inconsistent differences. The anomalous results obtained for savoy in 1947 did not recur and this crop behaved similarly to all others tested in 1948.

The molybdenum content of dried material was determined using Piper and Beckwith's method (7). The results given in Table III show that deficiency effects were associated with very low levels of molybdenum, ranging from 0·01 to 0·25 p.p.m. of molybdenum in dried leaf material. Where molybdenum was added the foliage was found to contain from 1·33 to 6·29 p.p.m. of molybdenum. The stems and roots contained less molybdenum than the leaves. The results suggest that the old leaves contained more molybdenum

TABLE I.

YIELD DATA.

| Crop | Date Harvested | Portion Harvested | Mean Yield in Grams. per Beaker | | | |
|---|---|---|---|---|---|---|
| | | | —Mo | | +Mo | |
| | | | Fresh Wt. | Dry Wt. | Fresh Wt. | Dry Wt. |
| Alsike Clover .. | 26. 8.48 | Shoot (First Cut) | 117 | 22·4 | 275 | 59·4 |
| | 14.10.48 | Shoot (Second Cut) | 166 | 32·4 | 253 | 55·4 |
| | | Total | 283 | 54·8 | 528·0 | 114·8 |
| Red Clover .. | 12.10.48 | Shoot (First Cut) | 138 | | 435 (grown in clay pot). | |
| Lucerne .. .. | 26. 8.48 | Shoot (First Cut) | 89·0 | 20·3 | 148 | 33·3 |
| | 14.10.48 | Shoot (Second Cut) | 96·6 | 18·9 | 142 | 28·9 |
| | | Total | 185·6 | 39·2 | 290 | 62·2 |
| Pea .. .. | 26. 8.48 | Shoot | 84·0 | — | 95·0 | — |
| | | Peas | 19·0 | — | 25·5 | — |
| | | Total | 103·0 | | 120·5 | |
| Broad Bean .. | 26. 8.48 | Shoot | 127 | — | 101 | — |
| | | Pods | 83·0 | — | 70·0 | — |
| | | Beans | 18·0 | — | 35·0 | — |
| | | Total | 228 | | 206 | |
| Barley .. .. | 26. 8.48 | Shoot | 101 | — | 83·0 | — |
| | | Fruiting heads | 11·3 | — | 16·0 | — |
| | | Total | 112·3 | | 99·0 | |
| Perennial Rye Grass .. .. | 17. 9.48 | Shoot (First Cut) | 215 | 54·5 | 302 | 81·0 |
| | 14.10.48 | Shoot (Second Cut) | 64·9 | 11·9 | 86·2 | 164 |
| | | Total | 279·9 | 66·4 | 388·2 | 97·4 |
| Cocksfoot .. | 26. 8.48 | Shoot (First Cut) | 185 | 42·7 | 272 | 58·5 |
| | 17. 9.48 | Shoot (Second Cut) | 70·5 | — | 132 | — |
| | 14.10.48 | Shoot (Third Cut) | 81·2 | 16·3 | 99·1 | 19·2 |
| | | Total | 336·7 | | 503·1 | |
| Yorkshire Fog .. | 17. 9.48 | Shoot (First Cut) | 370·0 | 79·5 | 440·0 | 102·0 |
| Sugar Beet .. | 8.10.48 | Root | 89·0 | 20·6 | 181·0 | 36·0 |

than the young in cauliflower and celery but not in kale. Successive cuts of the clovers, lucerne and grasses showed a reduction in the molybdenum content of the shoot and a corresponding increase in the severity of the molybdenum deficiency symptoms.

TABLE II.

NITRATE CONTENT AS P.P.M. IN THE MORGAN'S EXTRACT OF PETIOLES OF CONTROL AND MOLYBDENUM-DEFICIENT PLANTS.

| Crop | Time of Sampling | Portion Sampled | —Mo | +Mo |
|---|---|---|---|---|
| Cocksfoot .. | 10.30 a.m. 16. 9.48 | Leaves | 160 | 80 |
| Kale .. .. | 11 a.m. 23. 6.48 | Petiole | 50 | 5 |
| | 8 p.m. 16. 7.48 | „ | 10 | Nil |
| Swede .. .. | 11 a.m. 23. 6.48 | „ | 200 | 60 |
| | 11 a.m. 4. 8.48 | „ | 160 | Nil |
| Swede (resown) | 15. 9.48 | „ | 210 | 20 |
| Savoy .. .. | 11 a.m. 24. 7.48 | „ | 150 | Nil |
| | 8 p.m. 16. 7.48 | „ | 360 | 70 |
| | 3 p.m. 12. 8.48 | „ | 30 | Nil |
| Cauliflower .. | 3 p.m. 6. 7.48 | „ | 80 | 2 |
| | 11 a.m. 29. 7.48 | „ | 200 | 5 |
| Sugar Beet .. | 11 a.m. 23. 6.48 | „ | 180 | 15 |
| | 8 p.m. 16. 7.48 | „ | 160 | 5 |
| | 11 a.m. 4. 8.48 | „ | 160 | Nil |
| | 20. 9.48 | Root | 40 | 20 |
| Celery .. .. | 11 a.m. 29. 7.48 | Petiole | 400 | 180 |
| Tomato .. .. | 11 a.m. 23. 6.48 | „ | 180 | Nil |
| | 3 p.m. 7. 8.48 | „ | 55 | 7 |
| Tomato (resown) | 15. 9.48 | „ | 220 | 45 |
| Tomato .. .. | 20. 9.48 | Root | 60 | 80 |

It is of interest to note that the lowest molybdenum content of the controls was recorded for the perennial rye grass which initially contained 1·33 p.p.m. of molybdenum, whereas in the molybdenum-deficient plants cocksfoot contained the least amount of molybdenum, namely 0·01 p.p.m.

*Osmotic Pressure Data.* It was found that the stomatal guard-cells of

various molybdenum-deficient leaves had a higher osmotic pressure than those of the control plants (Table IV). These results confirmed those obtained in 1947.

TABLE III.

THE MOLYBDENUM CONTENT OF CONTROL AND MOLYBDENUM-DEFICIENT PLANTS EXPRESSED AS PARTS PER MILLION OF DRY MATTER.

| Crop | Portion Analysed | | | +Mo | —Mo |
|---|---|---|---|---|---|
| Alsike Clover .. | 1st Cut; | (25/ 8/48) | Leaf and Flower | 5·60 | 0·08 |
| | ,, | ,, | Stem | 2·38 | 0·02 |
| | 2nd Cut; | (14/10/48) | Leaf and Flower | 3·49 | 0·05 |
| | ,, | ,, | Stem | 1·60 | 0·03 |
| Red Clover .. | 1st Cut; | (12/10/48) | Leaf and Flower | 2·85 | 0·06 |
| | ,, | ,, | Stem | 2·16 | 0·02 |
| Lucerne .. | 1st Cut; | (25/ 8/48) | Leaf | 5·95 | 0·17 |
| | ,, | ,, | Stem | 2·52 | 0·05 |
| | 2nd Cut; | (14/10/48) | Leaf | 4·76 | 0·09 |
| | ,, | ,, | Stem | 2·90 | 0·02 |
| Perennial Rye Grass | 1st Cut; | (18/ 9/48) | Whole Shoot | 1·33 | 0·09 |
| | 2nd Cut; | (14/10/48) | ,, ,, | 3·08 | 0·04 |
| Cocksfoot .. .. | 1st Cut; | (25/ 8/48) | Whole Shoot | 1·63 | 0·01 |
| | 2nd Cut; | (14/10/48) | ,, ,, | 2·02 | 0·03 |
| Yorkshire Fog .. | 1st Cut; | (19/ 9/48) | Whole Shoot | 4·20 | 0·07 |
| Marrowstem Kale .. | Lamina | (7/10/48) | Old | 3·46 | 0·10 |
| | ,, | ,, | Young | 4·70 | 0·13 |
| | Stem | ,, | | 1·55 | 0·08 |
| Swede .. .. | Lamina + Petiole | (7/11/48) | | 4·87 | 0·14 |
| | Root | ,, | | 1·54 | 0·12 |
| Cauliflower .. | Lamina + Petiole | (12/10/48 | Old | 2·18 | 0·02 |
| | ,, ,, | ,, | Young | 0·96 | — |
| Sugar Beet .. .. | Lamina + Petiole | ( 8/10/48) | | 4·49 | 0·21 |
| | Root | ( 8/10/48) | | 0·46 | 0·05 |
| Celery .. .. | Lamina + Petiole | ( 8/10/48) | Old | 6·29 | 0·25 |
| | ,, ,, | ( 8/10/48) | Young | 1·45 | 0·07 |

TABLE IV.

OSMOTIC PRESSURE VALUES FOR STOMATAL GUARD CELLS OF THE LEAVES OF CONTROL AND MOLYBDENUM-DEFICIENT PLANTS (23/6/48).

| Crop | Atmospheres | |
|---|---|---|
| | +Mo | —Mo |
| Kale .. .. .. | 6·16 | 9·52 |
| Swede .. .. .. | 6·16 | 8·40 |
| Tomato .. .. | 3·36 | 6·72 |
| Sugar Beet .. .. | 14·55 | 19·60 |

## DISCUSSION.

The general similarity of certain of the symptoms observed was notable and merits some comment. The frequent marginal scorching and rapid drying out of parchment-like areas of the leaves may indicate some connection between the water relations of the leaf and molybdenum metabolism. Additional support for this suggestion comes from the differences in osmotic pressure reported both in this and in an earlier paper (2). The distinction noted previously between plants like cauliflower, that show acute effects in the growing point or younger parts, and plants such as tomato, which appear able to maintain the growth of the shoot at the expense of older regions, was again suggested from results obtained here. Thus clovers, lucerne, pea, tomato and flax maintained shoot growth at the expense of older leaves, whereas the Brassicae, kale, swede and cauliflower frequently showed more acute effects in or near the growing point than in the older leaves. This difference may possibly be due either to the relative mobility of, or the relative requirement for, molybdenum in different parts of the plant and further experiments are necessary to establish which may be the cause.

The visual symptoms suggestive of nitrogen deficiency, seen mainly in sugar beet, celery, grasses and cereals, were interesting, as molybdenum is probably a catalyst in some phase of nitrogen metabolism in the higher plants. The large accumulation of nitrate produced by lack of molybdenum was associated with depletion of amino acids (reported elsewhere (4))—a result consistent with the view expressed above. Nitrate accumulation has also been found by Mulder (6), Wilson (9) and by Stout and Meagher (8) who also showed that the pale mottled areas of molybdenum-deficient tomato leaves contained less molybdenum than the darker areas, while Wilson and Waring (10) found that the pale mottled areas in molybdenum-deficient cauliflower leaves contained higher nitrate concentrations than the darker green areas. Unpublished observations made by us also show that excess of molybdenum may produce effects in cauliflower resembling toxicity of urea or ammonium compounds.

The effects of true nitrogen deficiency, however, differ from those of molybdenum deficiency in certain respects. Thus molybdenum deficiency does not induce the tinting associated with nitrogen deficiency and the latter does not cause the characteristic injury to leaves or growing points; the cause of this breakdown may perhaps be sought in the accumulation of nitrate or depletion of other nitrogenous metabolites—this problem is receiving attention. The possibility that molybdenum may be essential for some stage of meristematic differentiation must also be considered.

During these experiments the severity of deficiency symptoms in some plants, particularly Brassicae and clovers, seemed to fluctuate in a periodic manner. No satisfactory explanation can be offered at present but possible causes might be minor changes in the molybdenum available to the plants, due to error in technique, the liberation of molybdenum from senescent or necrotic tissues, or possible variation in requirements for molybdenum at different stages of development.

The chemical analyses confirmed that plants showing molybdenum deficiency symptoms contained very much less molybdenum than the control plants. Although kale and swede are very sensitive to the lack of molybdenum and might be expected to have a high molybdenum requirement it is seen from Table III that less sensitive crops, such as celery, sugar beet and lucerne, contain more molybdenum. The analytical results agree with those previously

obtained (3) for mustard, tomato and cauliflower in that the leaves contain more molybdenum than the stems, but the distribution between old and young leaves is not consistent.

## SUMMARY.

1. The following plants were grown in molybdenum deficient sand culture: Alsike clover, red clover (Dorset marl), lucerne (Grim strain), pea (Onward), dwarf bean (Masterpiece), broad bean (Long pod), barley (Plumage archer), rye, oat (Red Algerian), perennial rye grass (Aberystwyth S.23), cocksfoot (Aberystwyth S.37), Yorkshire fog (*Holcus lanatus*) and purple Molinia (*Molinia Caerulea*), kale (Marrowstem), swede (Purple top), sugar beet, flax (Liral prince), celery (Giant white), tomato (Market king).

2. Marked visual effects attributed to molybdenum deficiency were seen in Alsike clover, red clover, lucerne, oat, kale, swede, sugar beet, flax and tomato.

3. Symptoms resembling nitrogen deficiency were observed in pea, dwarf bean, broad bean, barley, rye, perennial rye grass, cocksfoot, Yorkshire fog, sugar beet and celery.

4. Severe molybdenum deficiency symptoms were accompanied by marked dimunition in crop yields.

5. Nitrate determinations on leaf tissue extracts again confirmed the marked accumulation of nitrate in molybdenum-deficient plants.

6. A tentative method is described for the preparation of ferric citrate free from manganese, zinc, copper and gallium as well as from molybdenum.

7. The effects of molybdenum deficiency in relation to the nature and distribution of the symptoms and to certain aspects of nitrogen metabolism are discussed.

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## APPENDIX.

### *Visual Symptoms of Molybdenum Deficiency*

The approximate time, in days after planting or sowing, necessary for the development of molybdenum deficiency symptoms under the conditions of this experiment is given in brackets.

### Leguminosae.

**Alsike.**

Growth markedly reduced; leaflets pale green, margins greyish then curling forward; followed by pale brown or buff marginal scorch (43 days) and intervenal hatching; finally, complete withering of leaflets.

**Red Clover.**

Growth markedly reduced; individual leaflets becoming dull green and paling at margins, margins curling forward, becoming brown and scorched (43 days).

**Lucerne.**

Growth reduced; upper expanded leaflets dull green with characteristic grey hatching (26 days) near leaf tip; margins pale, curling forward with white or buff coloured tip marginal scorch finally involving whole leaf (35 days).

**Pea.**

Growth slightly reduced. No symptoms which could be regarded as diagnostic of molybdenum deficient plants were observed, except that the older leaves withered more rapidly than in the controls and were paler and more yellow; pods and seeds were reduced in number.

**Dwarf Bean.**

Good growth; prophylls appeared smaller than in the controls and later leaves became pale yellow-green; no distinctive foliar symptoms.

**Broad Bean.**

Growth as for control plants; leaves paler, yellowish green and appearing nitrogen-deficient.

### Gramineae.

**Barley.**

No reduction in growth; leaf tips cream or pale brown colour (17 days); foliage lustreless, dull greyish green and more susceptible to mildew than control.

**Rye.**

Growth reduced; foliage pale yellowish green, grey later, leaves white tipped with bleached irregular areas along lamina (85 days).

**Oat.**

Growth reduced; foliage pale green with grey lesions along lamina becoming orange red flecks, followed by intervenal bleaching and reddish scorch of older leaves (80 days) starting from leaf tips (frequently scorched to give orange-brown coloured appearance).

**Perennial Rye Grass.**

Growth reduced; foliage pale green suggestive of nitrogen deficiency; considerable withering of older leaves.

**Cocksfoot.**

Growth reduced; foliage pale yellow-green suggestive of nitrogen deficiency; considerable withering of older leaves.

**Yorkshire Fog.**

Growth reduced; foliage pale yellowish green, leaf tips becoming bleached (80 days) and subsequently withering.

### Cruciferae.

**Kale.**

Growth stunted; pale yellow-green leaves (13 days) at first, followed by diffuse bright yellow green intervenal mottling and cupping of margin (19 days); cupping tended to diminish, leaf margin became pale greyish green and then limp (48 days), limp region rapidly drying out to a buff coloured scorch (this change often occurring in a day or two)—the marginal region becoming papery, shrivelled and incurled; scorching also occurred in extensive intervenal areas. One

plant later in season (Aug-Sept.) showed a defective growing point; the young leaves were necrotic but the plant recovered and three shoots grew out from the main axis.

**Swede.**

Growth markedly reduced, leaves yellow-green (19 days) soon after germination, followed by grey tinting and sudden collapse of the leaf margin which became scorched and inrolled simultaneously (25 days), finally complete leaf withered; cotyledons persisting green for longer period; subsequent leaves developed distinct marginal cupping, pale green tint, diffuse intervenal mottling followed by buff coloured marginal scorching; later (July-Aug.) younger expanded leaves distorted, narrow and partially lacking in lamina with brown and withered leaf tips; lamina sometimes absent; roots produced were small. Severe deficiency killed some seedlings in 29 days.

**Savoy Cabbage.**

19 days after germination seedlings were exhibiting the typical molybdenum deficiency symptoms described previously (2), but recovered later.

## Other families.

**Sugar Beet.**

Growth reduced; pale yellowish green mottling occurring in the expanding leaves; older leaves yellow-green with rounded margins and faint pink marginal tinting (suggestive of nitrogen deficiency), becoming curled forward with buff coloured scorch commencing from near leaf tip and spreading towards the base and finally shrivelled (43 days).

**Celery.**

Growth dwarfed; foliage chlorotic, yellow or bright yellow-green, as if nitrogen deficient.

**Flax.**

Growth slightly reduced; shoot pale dull green colour, upper leaves pale, yellowish green at the tips (32 days) and later brown tip scorching (39 days), subsequently involving whole lamina; finally all lower and middle leaves becoming completely withered and a pale buff colour (80 days); at flowering wilting of the pedicels occurred about 2-5 cms. behind the flower or flower bud whilst the intervening regions remained green, wilting followed by withering of whole inflorescence.

**Tomato.**

Seedlings were transplanted into sand on 27.5.48. On 12.6.48 they were showing the typical deficiency symptoms described previously (2).

# MANURIAL EXPERIMENTS ON VEGETABLE CROPS.

## XIV. EFFECTS OF FARMYARD MANURE AND OF VARIOUS FERTILISER TREATMENTS ON THREE VARIETIES OF POTATO—SEASON 1948.

By T. WALLACE and E. CATLOW.

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The present report concerns the second of two successive potato crops, grown in 1948, on the continuous vegetable manurial plots. The crop was the tenth of the series begun in 1940.

In both 1947 and 1948 the same three varieties of potato, Majestic, Kerr's Pink and Red King, were grown for comparative purposes under the various manurial treatments, as an important aspect of the trials was to study the behaviour of these three sorts under conditions of different mineral nutrient status. The field trials were linked with chemical investigations. The three varieties used had been selected for the particular study as previous experience with them had indicated that they reacted differently to mineral deficiencies and excesses, Majestic appearing to be a 'resistant' variety in this respect and the other two easily affected by unfavourable conditions.

Moreover, Kerr's Pink had shown itself to be particularly suited for visual diagnosis studies, whilst Red King is unsatisfactory for this purpose as it shows rapid collapse of leaves and haulm and poor leaf patterns. These related studies also provide the reason for growing two successive crops of potatoes on the plots.

The details of the 1947 experiment are given in the 1947 Report (1).

In 1948 the planting date was earlier than in 1947, viz. April 16th as against April 25th, and both the farmyard manure and fertilisers were applied just prior to planting and after the ridges had been split in preparation for planting. The planting system for the three varieties differed from 1947, each variety being planted in three adjacent rows in each plot, Majestic on the west side, Kerr's Pink in the centre and Red King on the east. This arrangement had been found necessary from the previous year's experience to simplify planting and harvesting operations.

Weather conditions during the summer months were particularly favourable for vegetative growth, rainfall being abundant, and this fact seemed to decrease the magnitude of the nitrogen deficiency effects usually seen on the P.K. treated plots.

### Results.

*Field observations.* Vigour markings made on June 9th and August 5th illustrate well the relative growth conditions of the haulms during the season. These were as follows :—

Possible marks 15—each value the total of three plots.

| | *N.P.K.* | *F.Y.M.* | *N.P.* | *N.K.* | *N only* | *P.K.* | *N.P. KCl* | *N.P. NaCl* |
|---|---|---|---|---|---|---|---|---|
| 9/6/48 .. | 12 | 10½ | 10 | 8½ | 8 | 5 | 13 | 12 |
| 9/8/48 .. | 12 | 11½ | 6 | 11 | 9 | 6 | 14 | 13½ |

It will be noted that on both occasions the treatments fell into two distinct groups and that these groups were identical for the two dates, with one exception, a change over of the treatments N.P. and N.K. This latter feature is a common one on these plots and is due mainly to the fact that growth begins very slowly on the N.K. plots, due to the low phosphate status and the particular needs of crops for phosphorus in the early stages of growth. On the other hand potassium deficient plants (N.P. treatment) begin well, but with a crop like potatoes, with a high requirement for potassium, the early growth is followed by collapse of the vegetative organs.

The continued vigour of the crops receiving chloride treatments was again notable but it must be remembered that these plots are not strictly comparable with the others on which the treatments have been of longer duration.

On the main series of plots the usual features were shown on the foliage characters, noteworthy seasonal points being the greener appearance of the foliage on the P.K. plots than is usual, the more severe and earlier symptoms of potassium deficiency on the N.P. than on N. only plots, and the relative severity of magnesium deficiency effects on the plots. Markings made on Kerr's Pink give a good picture of this last point. Results were as follows :

| *N.P.K.* | *P.K.* | *N.K.* | *F.Y.M.* | *N. only* | *N.P.* | *N.P. KCl* | *N.P. NaCl* |
|---|---|---|---|---|---|---|---|
| 16 | 15 | 11 | 1 | 0 | 0 | 11 | 0 |

18 = severe ; 15 = medium ; 9 = slight ; 3 = trace.

In the chloride series, chloride injury was noted from both treatments as an edge scorching of the younger leaves. Vigour, however, remained unimpaired from this cause. Slight magnesium deficiency effects were also noted on the KCl plots. The most notable point, however, was the appearance for the first time in the course of the experiments of appreciable potassium deficiency on the NaCl plants, which was most marked on Kerr's Pink. The yields given below will show that this deficiency caused a substantial decrease in the crop.

The three varieties were affected to different extents by the various adverse treatments, Majestic invariably showing less effects than the other two varieties, and whilst Kerr's Pink frequently showed more marked effects than Red King on the leaves, the haulm of the latter variety tended to collapse first under extreme conditions, particularly on potassium deficient plots.

Leaf samples were collected for chemical examination from all the plots on August 5th and, so far, data for these are available only for the tissue test method. These latter results show close agreement with the records of the field observations and, in particular, show the greater effects produced by deficiencies of potassium and magnesium and by excess of chloride on the two varieties Kerr's Pink and Red King. The low potassium status of the plants on the NaCl plots is clearly shown, and the results for Kerr's Pink are especially low.

The full chemical results will be reported later.

*Yields.* The summarised data for yields are given in Tables I, II and III.

TABLE I.

Total Yields (Tons per Acre).

| Manurial Treatment | N | N.P.K. | P.K. | N.K. | N.P. | F.Y.M. | Mean | N.P. KCl * | N.P. NaCl * |
|---|---|---|---|---|---|---|---|---|---|
| *Variety.* | | | | | | | | | |
| Red King .. .. | 7·53 | 12·48 | 6·69 | 11·11 | 7·02 | 10·55 | 9·23 | 15·35 | 11·41 |
| Majestic .. .. | 7·26 | 13·52 | 7·77 | 12·29 | 8·12 | 12·03 | 10·17 | 17·01 | 15·11 |
| Kerr's Pink .. | 6·16 | 15·05 | 6·97 | 11·41 | 6·24 | 13·58 | 9·90 | 19·85 | 15·24 |
| Mean .. .. | 6·98 | 13·68 | 7·14 | 11·60 | 7·13 | 12·05 | 9·76 | 17·40 | 13·92 |
| Order .. .. | 6 | 1 | 4 | 3 | 5 | 2 | | | |

*Significant Differences required* (1% point)

Manurial Means over all varieties = 2·85

Differences due to Variety and Interaction V × M are not significant. Total yields follow same order as for 'ware yields,' with the exception 'Omit K' and 'N only' treatments.

TABLE II.

Ware Yield (Tons per Acre).

| Manurial Treatment | N | N.P.K. | P.K. | N.K. | N.P. | F.Y.M. | Mean | N.P. KCl * | N.P. NaCl * |
|---|---|---|---|---|---|---|---|---|---|
| *Variety.* | | | | | | | | | |
| Red King .. .. | 7·29 | 11·97 | 6·43 | 10·85 | 6·43 | 10·23 | 8·87 | 14·76 | 10·78 |
| Majestic .. .. | 7·13 | 13·20 | 7·66 | 12·11 | 7·87 | 11·84 | 9·97 | 16·50 | 14·81 |
| Kerr's Pink .. | 5·84 | 14·36 | 6·77 | 11·25 | 5·76 | 13·28 | 9·54 | 19·07 | 14·62 |
| Mean .. .. | 6·75 | 13·18 | 6·94 | 11·40 | 6·69 | 11·79 | 9·46 | 16·78 | 13·40 |
| Order .. .. | 5 | 1 | 4 | 3 | 6 | 2 | | | |

*Significant Differences required* (1% point)

Manurial Means over all Varieties = 2·182

Varietal „ „ „ Manures = 0·891

Interaction M × V Means of 3 replicates = 2·183

* These treatments not strictly comparable with main series.

TABLE III.

Ware Yield as Percentage of Total Yield.

| Manurial Treatment | N | N.P.K. | P.K. | N.K. | N.P. | F.Y.M. | Mean | N.P. KCl | N.P. NaCl |
|---|---|---|---|---|---|---|---|---|---|
| *Variety.* | | | | | | | | | |
| Red King .. .. | 96·8 | 95·9 | 96·0 | 97·6 | 91·6 | 96·9 | 95·80 | 96·2 | 94·2 |
| Majestic .. .. | 98·15 | 97·6 | 98·6 | 98·5 | 97·0 | 98·4 | 98·05 | 97·0 | 98·0 |
| Kerr's Pink .. | 94·8 | 95·4 | 97·3 | 98·6 | 92·3 | 97·8 | 96·03 | 96·1 | 95·9 |
| Mean .. .. | 96·67 | 96·28 | 97·37 | 98·23 | 93·85 | 97·77 | 96·69 | 96·40 | 96·27 |

Inspection of the data shows that in the main series, the plots both as regards total yields and ware fall into two very distinct groups, these being as for the vigour markings on August 5th. The results for the chloride plots show the markedly lower yields on the NaCl plots, associated with their lower potassium status.

Statistical analysis applied to the data on the main series of plots shows the following points judged at the 1% point of significance.

*Total yields*: Treatments N.P.K., F.Y.M. and N.K. > P.K., N.P. and N only.

*Ware*: as for total yields.

*Varieties*: differences are not significant for total yields, but for ware Majestic > Red King.

In interactions of varieties and manures, differences are significant for ware yields and Kerr's Pink gives higher yields than Red King for the high yielding treatments N.P.K. and F.Y.M. Notable points in relation to these interactions are that results for total yields and ware are in similar directions and that Kerr's Pink tends to give the highest yields under favourable treatments but may give the lowest yields under unfavourable treatments, particularly so when potassium is deficient.

## SUMMARY.

The results of a manurial experiment carried out in 1948 on potatoes on a series of continuous manurial plots are reported.

The experiment was a repetition of a similar trial carried out on the same plots in 1947.

Three varieties were used, viz. Majestic, Kerr's Pink and Red King. They were selected for the study in view of their differential responses to unfavourable conditions of mineral nutrient status.

Data were obtained relating to growth features, mineral status of leaves and yields of tubers.

The treatments in the main series fell into two distinct groups as regards yields, low levels of potassium and nitrogen resulting in low yields.

Significant differences were shown for yields of ware and for the interaction of varieties and fertiliser treatments in respect of ware yields.

Kerr's Pink gave high yields of tubers under favourable fertiliser treatment but tended to give the lowest yields when either nitrogen or potassium was deficient.

Potassium deficiency was observed for the first time in these experiments on plots receiving common salt *vice* muriate of potash and the deficiency was associated with a large decrease in yield.

## ACKNOWLEDGMENT.

The chemical data referred to in these experiments were obtained by Dr. D. J. D. Nicholas, to whom thanks are due.

### REFERENCE.

(1) Nicholas, D. J. D. and Catlow, E. (1947). Manurial experiments on vegetable crops. XIII. Effects of farmyard manure and other manurial treatments on potato and cauliflower. Seasons 1946 and 1947. *Ann. Rept. Long Ashton Res. Sta.*, 118–125.

# MANURIAL EXPERIMENTS ON VEGETABLE CROPS.

## XV. EFFECTS OF FARMYARD MANURE AND OTHER MANURIAL TREATMENTS ON POTATO. SEASON 1948.

By T. WALLACE and E. CATLOW.

The potato crop grown on these plots in 1948 was the tenth in the series of continuous vegetable crops. The two previous crops which were reported upon in the 1947 report (1) were potato, 1946, and cauliflower, 1947. The three varieties grown were Majestic, Kerr's Pink and Red King, and the cropping plan was identical with that on the parallel series of plots comparing the effects of dung and various inorganic fertiliser treatments (p. 91). As in previous years the crops were used for chemical investigations to supplement the yield data.

The manurial treatments given were as described for the 1946 potato crop, with one modification, and the sources of the bulky organic manures were the same as for that year. The modification consisted in the alteration of the rate of calcined kieserite, the MgO content of which material had fallen to 16% as the result of long storage. In 1948 this material was applied in treatment H at the rate of 2 cwt. per acre *vice* 1 cwt. previously used. The bulky manures were all applied on April 16th and non-bulky manures and fertilisers on April 19th. Planting was also carried out on the latter date. Harvesting took place on September 29th.

**Results.**

*Field Observations.* Visual records of vigour and foliage characters were made on four occasions between June 10th and September 6th. The weather conditions during 1948 were consistently favourable for vegetable growth and as a result vigour was generally good on all plots. The following data will indicate the relative vigour on the various plots during the active growing season.

TABLE I.

Vigour Marking—3 Plots each Treatment, Maximum Points Possible, 15.

| Date | A F.Y.M. | B ½ F.Y.M. + ½ N | C Veg. Compost + ½ N | D 2N (Hoof) P.K. | E N.P.K. inorganic | F N.P.K. (K as potash salts) | G Straw/ Sewage sludge Compost | H N.P.K. + Mg |
|---|---|---|---|---|---|---|---|---|
| 10/6/48 | 9 | 11 | 12½ | 12 | 12½ | 11 | 10 | 12 |
| 19/7/48 | 13 | 12½ | 12 | 13½ | 12 | 13½ | 11 | 12½ |
| 5/8/48 | 13½ | 12½ | 12 | 13½ | 12½ | 12½ | 11½ | 12 |
| 6/9/48 | 13½ | 13½ | 13 | 15 | 12½ | 12½ | 11½ | 12½ |

The only notable points in comparing the vigour data are that treatment D plots carried the most vigorous haulm and G plots the least vigorous.

Certain colour differences were noticeable throughout the season, mainly reflecting the status of nitrogen and potassium and the effect of excess chloride in treatment F.

Records made on August 5th provide a representative picture.

Treatment A—slightly pale to pale green; B—slightly pale green; C—slightly pale to medium green; D—dark green; E—medium green; F—slightly pale to medium green; G—medium green; H—medium green.

It will be seen that on the plots receiving F.Y.M. (A and B), vegetable compost (C) and potash salts (F), the foliage tended to be pale, whilst on the hoof plots (D) it was dark green. In previous years foliage on the straw/sludge compost plots (G) has been markedly pale green, due to a low nitrogen status, but in 1948 potassium deficiency was in evidence and the leaves were a darker green colour. This also occurred to some extent in treatment C. Slight symptoms of magnesium deficiency developed on some of the plots and the incidence of these in relation to the manurial treatments is shown in the following records made on August 5th.

| Treatments | *A* | *B* | *C* | *D* | *E* | *F* | *G* | *H* |
|---|---|---|---|---|---|---|---|---|
| | 1 | 6 | 5 | 8 | 13 | 2 | 0 | 0 |

18 = severe; 15 = medium; 9 = slight; 6 = very slight; 3 = trace.

It will be seen that the symptoms were only appreciable in treatments E and D and were negligible or absent from the plots receiving F.Y.M. (A), N.P.K. (potash salts) (F), straw/sludge compost (G) and N.P.K. + Mg (H).

A point of special interest during the season was the appearance of potassium deficiency symptoms on certain plots for the first time during the course of the experiments. These were mainly confined to the variety Kerr's Pink, which has also shown itself to be highly susceptible to this deficiency in the deficiency trial plots. (see p. 91). The most severe symptoms developed in treatment G (straw/sludge compost) and were also present in treatments H (N.P.K. + Mg) and C (vegetable compost and N). The first leaf symptoms were noted on August 5th. The low potassium status of the foliage on these plots was later confirmed by chemical tissue tests when the levels recorded for Kerr's Pink were as follows: G—nil, low, nil; H—medium —, low, low +; C—medium + on all three plots.

The result for G is of particular importance as it shows that the composting of sewage sludge with straw has not raised the potassium status of the sludge sufficiently to provide a satisfactory potash manure. The low potassium status in H is most probably due to antagonism between magnesium and potassium. In C, the deficiency doubtless arises from the fact that many vegetables contain much lower amounts of potassium than the potato and hence the composts made from their waste materials do not supply sufficient quantities of the element.

*Yields.* Data relating to total yield, ware and percentage ware in total yield are given in Tables II, III and IV.

TABLE II.

TOTAL YIELD (TONS PER ACRE).

| Manurial Treatment | A | B | C | D | E | F | G | H | Mean |
|---|---|---|---|---|---|---|---|---|---|
| *Variety* | | | | | | | | | |
| Red King .. .. | 15·69 | 14·06 | 10·53 | 14·14 | 12·27 | 10·39 | 10·04 | 11·09 | 12·28 |
| Majestic .. .. | 15·45 | 16·23 | 13·96 | 16·09 | 15·93 | 15·11 | 11·41 | 14·33 | 14·82 |
| Kerr's Pink .. .. | 18·78 | 16·42 | 13·93 | 17·22 | 13·96 | 14·76 | 11·39 | 14·25 | 15·07 |
| Mean .. .. .. | 16·64 | 15·57 | 12·80 | 15·82 | 14·05 | 13·42 | 10·95 | 13·22 | 14·06 |
| Order .. .. .. | 1 | 3 | 7 | 2 | 4 | 5 | 8 | 6 | |

*Manurial Means over all Varieties* (SE = 0·581)
Significant Difference required 1%—2·446 ; 5%—1·762.
*Varietal Means over all Manures* (SE = 0·336)
Significant Difference required 1%—1·303 ; 5%—0·969.

TABLE III.

WARE YIELD (TONS PER ACRE).

| Manurial Treatment | A | B | C | D | E | F | G | H | Mean |
|---|---|---|---|---|---|---|---|---|---|
| *Variety.* | | | | | | | | | |
| Red King .. .. | 15·19 | 13·68 | 10·12 | 13·88 | 11·99 | 10·04 | 9·62 | 10·77 | 11·91 |
| Majestic .. .. .. | 15·19 | 16·02 | 13·82 | 15·97 | 15·77 | 14·97 | 11·25 | 14·14 | 14·64 |
| Kerr's Pink .. .. | 18·38 | 16·13 | 13·66 | 16·96 | 13·72 | 14·52 | 10·82 | 13·93 | 14·76 |
| Mean .. .. .. | 16·25 | 15·28 | 12·53 | 15·60 | 13·83 | 13·18 | 10·56 | 12·95 | 13·77 |
| Order .. .. .. | 1 | 3 | 7 | 2 | 4 | 5 | 8 | 6 | |

*Manurial Means over all Varieties* (SE = 0·583)
Significant Difference required 1%—2·454 ; 5%—1·768.
*Varietal Means over all Manures* (SE = 0·308)
Significant Difference required 1%—1·188 ; 5%—0·884.

TABLE IV.

WARE YIELDS AS PERCENTAGE TOTAL YIELDS.

| Manurial Treatment | A | B | C | D | E | F | G | H | Mean |
|---|---|---|---|---|---|---|---|---|---|
| *Variety.* | | | | | | | | | |
| Red King .. .. | 96·81 | 97·29 | 96·10 | 98·16 | 97·71 | 96·63 | 95·81 | 97·11 | 96·96 |
| Majestic .. .. .. | 98·32 | 98·71 | 98·99 | 99·25 | 98·99 | 99·07 | 98·59 | 98·67 | 98·83 |
| Kerr's Pink .. .. | 97·87 | 98·23 | 98·06 | 98·49 | 98·28 | 98·37 | 94·99 | 97·75 | 97·97 |
| Mean .. .. | 97·65 | 98·13 | 97·72 | 98·65 | 98·43 | 98·02 | 96·47 | 97·84 | 97·84 |

It will be seen from the data that yields, both for total crop and ware, were high throughout and that percentage ware was also high for all treatments. It is notable in both yield tables that the treatments C, G, H, where potassium status was low, produced the lowest yields, and it is also of interest that the highest yields were obtained in treatment A (F.Y.M.) although the haulm on these plots appeared to indicate a suboptimal level of nitrogen. This latter relationship has been noted previously for potatoes on these plots, but with brassica crops, requiring a generous supply of nitrogen, low yields result from the farmyard manure treatment.

Statistical analysis of the yield results showed the following points as the 1% level of significance.

*Total yield :* A > C, E, F, G, H ; D > C, G, H ; B, E, F > G.
*Ware :* A, D > C, F, G, H ; B > C, G ; E, F > G.
*Varieties :* for both total yields and ware—
Kerr's Pink and Majestic > Red King.
*Ware as percentage Total Yield :* no significant effect.

## SUMMARY.

Three varieties of potato, Majestic, Kerr's Pink and Red King, were grown as the tenth crop in a manurial experiment, comparing the effects of various bulky and non-bulky organic manures and inorganic fertilisers.

Most of the treatments produced vigorous growth of haulm. There were slight differences in the colour of the foliage due to differences in nitrogen and potassium status and to excess chloride.

Potassium deficiency symptoms occurred on plots receiving the treatments straw/sludge compost, N.P.K. + Mg fertiliser, and vegetable compost + N. The variety Kerr's Pink was most affected and Majestic least. Low potassium status was confirmed by chemical tissue tests.

Slight symptoms of magnesium deficiency occurred and were most in evidence on plots receiving an inorganic N.P.K. fertiliser.

Yields were generally high; they were highest on the plots receiving F.Y.M. and lowest on the plots on which potassium deficiency effects were observed.



REFERENCE.

(1) Nicholas, D. J. D. and Catlow, E. (1947). Manurial experiments on vegetable crops. XIII. Effects of farmyard manure and other manurial treatments on potato and cauliflower. Seasons 1946 and 1947. *Ann. Rept. Long Ashton Res. Stn.* 118.


# THE RELATION BETWEEN THE WARING BLENDOR METHOD AND OTHER PROCEDURES FOR THE DIAGNOSIS OF THE MINERAL STATUS OF CROP PLANTS.


By D. J. D. NICHOLAS.


Rapid chemical tissue tests have been successfully used for the diagnosis of the mineral status of crop plants. Although they are less precise than the full chemical methods of analysis, the fact that they are quick in operation and that several can be made within a short space of time tends to outweigh that disadvantage.

In addition to the use of diffusion methods (9), macerators have been employed to comminute tissues for the extraction of nutrients from fresh plant material. The simplest procedure of this kind was used by Carolus (1, 2) who ground fresh plant tissues in a mortar in the presence of carbon and 2% acetic acid. Tests for K, Mg, Ca, P and nitrate N were made on the clarified extract. Hester proposed the use of the Waring blendor for the extraction of nutrients from 5 gm. of fresh tissue, using 100 ml. of acetate buffer (pH 4·8) in the presence of carbon. The time for maceration and the amount of charcoal required varied with the type of crop plant used (5). The blendor consists of a glass vessel fitted at the base with four stainless steel blades on a steel pivot which revolves at high speed. Wolf has used a similar procedure for the extraction of K, Mg, Ca, P, and nitrate N from bean, pea, tomato and asparagus (10, 11). Peech and English (7) have suggested that their microchemical tests for soil nutrients might be applicable to testing Waring blendor extracts for mineral nutrients, although they showed that organic matter present in the extracts interfered with some of the chemical

tests ; in particular, the Nessler test for ammonia (8). Harrington (4), using a similar procedure to that of Peech and English (8) (details of which are not given in either paper), showed that Waring blendor extracts of the stem portions of spinach and bean were good indicators of the major nutrients present in the tissues but they emphasised the importance of the study of the seasonal drift and of the interpretation of results in relation to soil type.

This paper gives preliminary data on the use of the Waring blendor for the extraction of K, Mg, Ca, P, Cl, and Mn (the last two when present in excess) from a variety of crop plants grown at various centres. The crops include potato, cauliflower, broad bean, sugar beet, linseed, oat, barley, and apple. In addition comparisons are made with other diagnostic methods, including visual diagnosis and ash analysis of the crop plants, as well as yield results.

## EXPERIMENTAL.

### Materials.

In Table I are given details of site, soil characters, crops grown and nutritional problems occurring at the various centres.

TABLE I.

| Centre | Soil characters | Crop Variety | Nutritional Problems |
|---|---|---|---|
| A<br>*Long Ashton.*<br>Expt. 1. | Calcareous sandy loam derived from red drift overlying Keuper Marl. | Potato—Kerr's Pink<br>Majestic*<br>Red King* | N, P (trace), K, Mg deficiencies. |
| | | Cauliflower—Majestic | N, K, Mg deficiencies. |
| | | Broad Bean* | K deficiency. |
| | | Linseed* | N, K deficiencies. |
| | | Sugar Beet* | N, K, Mn (trace) deficiencies. |
| | | Oat | N deficiency. |
| Expt. 2. | „ | Potato—Kerr's Pink<br>Majestic*<br>Red King* | N, P (trace) K, Mg deficiencies.<br>Cl toxicity. |
| Expt. 3. | Sand Culture. | Potato<br>Tomato | N, P, K, Mg, Ca deficiencies. |
| Expt. 4. | As for Expt. 1, but has calcareous parent material, mainly Keuper Marl. | Apple—Lane's Prince Albert | N, P (trace) K, Mg deficiencies. |
| B<br>Sutton Park, Birmingham. | Highly podsolised gravelly sand, mainly derived from glacial deposits of Bunter origin. | Potato—Majestic | N, P, K, Mg, Ca deficiencies.<br>Mn toxicity. |

Detailed descriptions of the layout of the experiments at Centres A and B have been given in previous papers (9).

* Chemical data, including Waring blendor results, for these crops are available for consultation at Long Ashton.

**Methods for Extraction and Testing of Nutrients for Rapid Tests and Ash Analysis.**

*Selection of Plant Parts.* Details of plant parts chosen for testing are given in previous papers (9). The importance of taking morphologically similar plant portions for testing has been emphasised.

*Preparation of Test Samples.* For the diffusion method of extraction the petiole portion of the leaf is usually taken.

For the Waring blendor procedure the following plant portions were selected for the extraction of the nutrients.

| | |
|---|---|
| Potato | Mid-stem leaves, leaflets near the tip of the petiole. |
| Broadbean | Mid-stem leaves. |
| Cauliflower | First upright leaves outside incurled leaves, midrib portions about halfway between leaf attachment and leaf tip. |
| Sugar beet | First upright leaves, petiole portions immediately adjacent to lamina. |
| Flax | Small stem portions near tip of plant. |
| Apple | 3rd and 4th leaves taken from the basal portion of well exposed leader shoots. |
| Barley, Oat | At young growth stage, the whole plant above ground ; later, internode adjacent to growing point. |

*Extraction of Nutrients.* Details of the extraction of the easily soluble nutrients, using the diffusion technique, have been reported (9).

The Waring blendor method used is as follows :—10 gm. of fresh plant tissue is cut into small portions on a glazed tile, using a sharp scalpel. These portions are put in the blendor together with 150 ml. of the acetate buffer solution (100 gm. of sodium acetate and 30 ml. glacial acetic acid in 1 litre of distilled water) and 2 gm. of Darco G 60 carbon. The material is macerated in the blendor for 2 minutes and the extract filtered through a Whatman filter paper into a 250 ml. graduated flask and then made up to volume with distilled water. The pH of the final solution approximates to 4·8. It is found that the first 20 ml. of filtrate may contain carbon, so this portion is poured back into the blendor for further filtration. The tissue extract should now be clear.

In instances of crop plants containing a high proportion of tannin, e.g. fruit trees, it may be necessary to increase the carbon to 5 gm. to facilitate the clarification of tissue extracts.

Suitable aliquots (usually 5 ml.) are taken for testing the nutrients. It may be necessary to make a further 1/5 dilution before testing, especially in the case of K and Mg.

*Chemical Tests Used.* The details of chemical tests used for determining K, Mg, Ca, P, Cl, and Mn, the last two when present in excess, have been given (9). To determine quantitatively the nutrients present in the extracts the Spekker Absorptiometer and the Biochem Absorptiometer were used. Suitable calibration diagrams were prepared and the nutrients present in the extract determined with reference to these.

## RESULTS.

**Purity of Carbon.**

American investigators (1, 2, 4, 5, 7, 8, 10, 11) have used Darco G 60 carbon for the clarification of tissue extracts. This charcoal was used in the present investigation because it is relatively free from mineral nutrients.

Several British carbons and the American Darco G 60 grade were tested for nutrients by homogenising 0·5, 1, 2, 3, 4 and 5 gm. respectively for various times, in the absence of plant tissues, using the Waring blendor procedure described previously.

The untreated British carbons contained 750—1,250 $\mu$g Mg, and 5,000—10,000 $\mu$g $PO_4$ per gm. The Darco G 60 product was relatively free from mineral nutrients, having 10 and 20 $\mu$g Mg and $PO_4$ per gm. respectively. Special washing of British carbons with hot HCl–NaCl mixtures reduced the impurities to levels found in the Darco product.

**Adsorption of Nutrients by Carbon.**

Previous workers do not appear to give data for the adsorption by carbon (if any) of the mineral nutrients extracted from plant tissues. To test this point several lots of the following nutrient solution were in turn blended in the presence of 2 gm. of carbon, using various time intervals, viz., 0·5, 1, 2, 3, 4, and 5 minutes respectively :—

| | | |
|---|---|---|
| $Ca(NO_3)_2$ | ≡ | 20 mg Ca and 61·8 mg nitrate N. |
| $KH_2PO_4$ | ≡ | 40 mg K and 5 mg $PO_4$. |
| $Mg(C_2H_3O_2)_2$ | ≡ | 2·5 mg Mg. |
| $KMnO_4$ | ≡ | 20 $\mu$g Mn. |
| $Al_2(SO_4)_3$ | ≡ | 25 $\mu$g Al. |

The above nutrient solution was made up to 150 ml. with the Morgan's solution and was homogenised in the blendor in the presence of carbon, filtered into a graduated flask, made up to 250 ml. and tested for mineral nutrients, using the rapid tissue test and also the full chemical analysis methods. The homogenising and chemical determinations made in duplicate show that, for the nutrients tested, very little adsorption takes place during blending. Thus the Darco carbon is suitable for clarification of extracts, as little of the inorganic nutrients are adsorbed. The agreement between the two methods of analysis is close.

**Time of Extraction of Nutrients.**

Experiments were made to determine the optimum time for the extraction of nutrients. The mid-stem leaves of tomato were selected for this purpose and were macerated in duplicate in the blendor, as described, for 0·5, 1, 2, 3, 4, and 5 minutes respectively. The results determined in duplicate are shown in *Fig.* 1 for K, Mg, Ca, and $PO_4$. The main point is that 2 minutes is

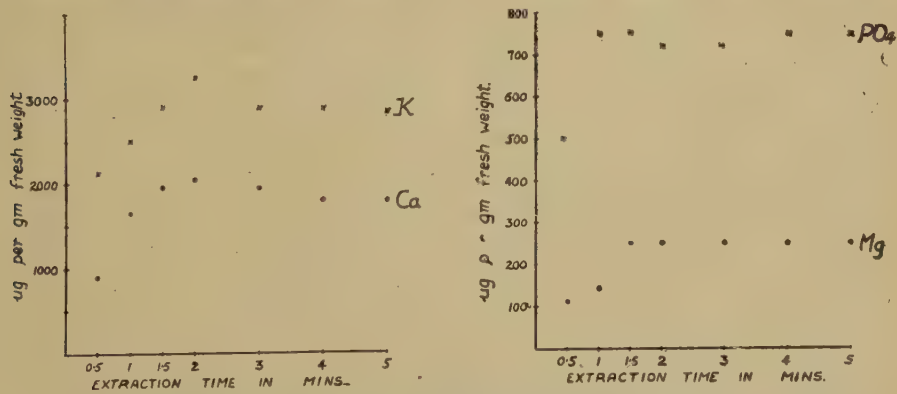


*Fig.* 1. The relation between the nutrients extracted from tomato leaves and time of comminution of the tissue in the Waring blendor.

the most appropriate minimum time for complete comminution of the tissue resulting in maximum extraction of the nutrients studied. Thus, for the investigations reported herein, 2 minute extraction was used.

*Standard Error of the Method.* Ten separate 10 gm. leaf portions of tomato were blended in turn and K, Mg, Ca, and P determined. The results when statistically treated showed that the deviation from the mean for the ten chemical determinations was within ± 10%.

## CENTRE A.

### Experiment 1.

At this centre a study was made of the nutrient status of potato (Kerr's Pink and Majestic), cauliflower (Majestic), broad bean, flax, sugar beet and oat at intervals during the growing season, using the diffusion, Waring blendor and, in some instances, ash analysis methods. In addition, visual symptoms were recorded and, whenever possible, yield data for the crops. As it will be impossible to include all the seasonal data, a selection is made to illustrate the correlations between the various methods.

In Tables II, III, and IV are given the results of the three methods of analysis, visual symptoms of the deficiencies and, whenever possible, yield data for the crops. The main points are as follows :—

*Potassium.* The most striking differences in effects of treatment are shown by potato and broad bean, where values, as determined by the three chemical methods in the nil and NP (omit K) plants are low. The three chemical methods, although giving values at different levels, roughly parallel one another in most of the crops studied. Symptoms of the deficiency were severe in potato and broad bean in the nil plot and particularly so in the NP (omit K) plot and are thus in accord with low chemical results for potassium. Moreover the yield figures for potato are significantly lower in the absence of K. That the three varieties of potato are equally susceptible to K deficiency is shown by symptoms, total yields and chemical results.

The susceptibility of these crops to the deficiency is also well shown by the data. Sugar beet, oat, and linseed on the other hand, did not show deficiency symptoms in the nil and NP (omit K) plots, and chemical values for the element are higher than in potato and broad bean.

*Magnesium.* Lower levels of the nutrient are recorded by the three methods in the NPK and particularly the PK (omit N) treatments in potato and cauliflower. These manurial effects on the Mg status are in agreement with previous observations (8). Varietal susceptibilities are shown by the chemical and visual methods ; thus Majestic is less affected than Kerr's Pink and Red King under NPK and PK manuring. The inter-relation of Mg and K is noteworthy ; when the last is deficient, as in NP treatment, the Mg level is higher than for the other treatments. Oat, sugar beet, linseed and broad bean do not appear to be susceptible to Mg deficiency under these conditions and this is confirmed by the adequate chemical values recorded.

*Calcium.* In all crops this nutrient is at satisfactory levels as shown by the chemical methods. This is in agreement with the presence of approximately 5% free lime in the surface soil. The cauliflower, potato, broad bean and linseed have higher values than sugar beet. The higher Ca levels when K is deficient are well shown in potato and cauliflower.

*Phosphorus.* Chemical results show that the NK (omit P) treatments

TABLE II.

RELATION BETWEEN THE DIFFUSION (D) AND WARING BLENDOR (WB) TESTS, ASH ANALYSIS (AA), YIELD DATA AND VISUAL SYMPTOMS IN POTATO (KERR'S PINK). (SAMPLED 13/8/48).

| Nutrient tested | Type of test | *Block I* A Nil | B FYM | C NPK | D Omit N | E Omit P | F Omit K | *Block II** A Nil | B FYM | C NPK | D Omit N | E Omit P | F Omit K | *Block III* A Nil | B FYM | C NPK | D Omit N | E Omit P | F Omit K |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| K | D | 150 | 1600 | 2150 | 2250 | 2200 | 100 | 450 | 2050 | 2000 | 1700 | 1700 | 150 | 200 | 1700 | 1950 | 2000 | 1600 | 150 |
| | WB | 450 | 7840 | 8250 | 9200 | 8500 | 500 | 640 | 9900 | 9840 | 8900 | 8800 | 400 | 450 | 8850 | 9900 | 8600 | 8600 | 450 |
| | AA | 1·17 | 4·01 | 4·49 | 4·63 | 4·67 | 0·72 | 1·46 | 4·70 | 4·95 | 4·43 | 4·22 | 1·25 | 1·28 | 4·4 | 4·90 | 4·72 | 4·0 | 0·81 |
| Mg | D | 80 | 50 | 40 | 30 | 70 | 70 | 100 | 80 | 90 | 70 | 80 | 90 | 80 | 60 | 40 | 25 | 40 | 80 |
| | WB | 390 | 375 | 300 | 160 | 350 | 400 | 510 | 490 | 490 | 375 | 375 | 520 | 400 | 350 | 270 | 140 | 270 | 600 |
| | AA | 0·80 | 0·64 | 0·41 | 0·36 | 0·61 | 0·83 | 1·01 | 0·88 | 0·87 | 0·75 | 0·71 | 0·92 | 0·80 | 1·04 | 0·46 | 0·26 | 0·71 | 0·83 |
| Ca | D | 1150 | 750 | 750 | 600 | 950 | 1600 | 1350 | 600 | 650 | 600 | 600 | 1400 | 1400 | 700 | 1300 | 750 | 800 | 1600 |
| | WB | 4450 | 3550 | 3350 | 3425 | 3775 | 4650 | 5000 | 3425 | 4075 | 3225 | 3375 | 4725 | 4750 | 3300 | 3625 | 3600 | 3875 | 4850 |
| | AA | 4·35 | 3·64 | 3·52 | 3·46 | 3·87 | 4·93 | 4·78 | 3·36 | 3·56 | 3·31 | 3·32 | 4·67 | 4·79 | 3·45 | 4·77 | 3·73 | 3·80 | 4·92 |
| P | D | 200 | 200 | 250 | 250 | 100 | 300 | 200 | 250 | 250 | 300 | 150 | 250 | 200 | 200 | 250 | 200 | 100 | 350 |
| | WB | 750 | 700 | 800 | 700 | 300 | 900 | 600 | 850 | 750 | 750 | 450 | 800 | 700 | 650 | 800 | 700 | 300 | 1000 |
| | AA | 0·70 | 0·68 | 0·80 | 0·74 | 0·52 | 0·95 | 0·61 | 0·89 | 0·73 | 0·74 | 0·51 | 0·89 | 0·70 | 0·68 | 0·80 | 0·74 | 0·47 | 0·95 |
| N | AA | 6·0 | 3·8 | 5·40 | 2·4 | 4·63 | 6·34 | 6·0 | 3·92 | 5·20 | 2·3 | 4·63 | 6·40 | 6·30 | 3·6 | 5·32 | 2·13 | 4·76 | 6·61 |
| Mn | D | 600 | 200 | 300 | 250 | 200 | 700 | 500 | 300 | 300 | 400 | 400 | 500 | 500 | 300 | 200 | 250 | 300 | 600 |
| | AA | 62 | 24 | 28 | 26 | 26 | 78 | 66 | 25 | 32 | 33 | 28 | 59 | 57 | 25 | 39 | 38 | 37 | 68 |
| Visual deficiency symptoms | | K | Nil | Mg | N.Mg | P | K | K | nil | nil | N | P | K | K | nil | Mg | N.Mg | P (trace) | K |
| Yield of tubers | | Signif. Difference in each block at 1% level: B, C, D, E > A, F. | | | | | | | | | | | | | | | | | |

* $MgSO_4$ applied to all plots in Block II.

Diffusion and Waring blendor data: K, Mg, Ca, $PO_4$, Cl as $\mu$g per gm. fresh weight.
Mn as $\mu g \times 10^{-3}$ per gm. fresh weight.

Ash analysis data: % $K_2O$, MgO, CaO, $P_2O_5$ and N in dry matter.
Mn as $\mu$g per gm. dry matter.

TABLE III.

Relation Between the Diffusion (D), Waring Blendor (WB) Tests, Visual Symptoms and Yield Data in Cauliflower.
(Sampled 22.9.48).

| Nutrient tested | Type of test | $A_1$ | $B_1$ | $C_1$ | $D_1$ | $E_1$ | $F_1$ | $A_2$* | $B_2$* | $C_2$* | $D_2$* | $E_2$* | $F_2$* | $A_3$ | $B_3$ | $C_3$ | $D_3$ | $E_3$ | $F_3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| K | D | 400 | 960 | 1740 | 1080 | 1430 | 420 | 640 | 1470 | 1060 | 1160 | 1460 | 350 | 280 | 1630 | 1540 | 1500 | 1560 | 420 |
| | WB | 1100 | 5500 | 6000 | 7250 | 5600 | 1000 | 1500 | 5000 | 5500 | 6500 | 7250 | 1200 | 1400 | 7000 | 7500 | 7500 | 8000 | 1250 |
| Mg | D | 40 | 40 | 20 | 20 | 40 | 50 | 160 | 120 | 120 | 120 | 120 | 180 | 40 | 30 | 30 | 10 | 40 | 40 |
| | WB | 100 | 75 | 50 | 25 | 75 | 100 | 500 | 450 | 375 | 375 | 450 | 575 | 100 | 75 | 50 | 25 | 75 | 75 |
| Ca | D | 1950 | 1200 | 1100 | 1270 | 1320 | 1950 | 1600 | 1220 | 1100 | 920 | 1370 | 1420 | 1570 | 1200 | 1350 | 1370 | 1000 | 1820 |
| | WB | 5925 | 4375 | 4425 | 4250 | 4500 | 6125 | 5500 | 4675 | 4550 | 4625 | 5425 | 6050 | 5750 | 4925 | 4250 | 4675 | 4925 | 5650 |
| $PO_4$ | D | 500 | 500 | 500 | 450 | 100 | 100 | 400 | 400 | 500 | 400 | 100 | 600 | 450 | 400 | 500 | 400 | 150 | 600 |
| | WB | 1250 | 1250 | 1500 | 1250 | 300 | 1500 | 1250 | 1500 | 1500 | 1500 | 300 | 1500 | 1250 | 1250 | 1250 | 1250 | 400 | 1500 |
| Mn | D | 800 | 300 | 400 | 500 | 500 | 900 | 800 | 200 | 400 | 400 | 500 | 800 | 800 | 300 | 600 | 600 | 500 | 900 |
| Visual deficiency symptoms | | K | nil | Mg | N.Mg | nil | K | K | nil | nil | N | nil | K | K | nil | Mg | N.Mg | nil | K |
| Yields of marketable "heads" | | Signif. Difference in each block at 1% level: C > A, B, D, E, F; B > A, D; E, F > A. | | | | | | | | | | | | | | | | | |

* $MgSO_4$ applied to all plots in Block II.

Diffusion and Waring blendor data: K, Mg, Ca, $PO_4$, Cl as $\mu$g per gm. fresh weight.
Mn as $\mu g \times 10^{-3}$ per gm. fresh weight.

TABLE IV.

Relation Between the Diffusion (D), Waring Blendor (WB) Tests and Visual Symptoms in Oat. (Sampled 24.6.48).

| Nutrient tested | Type of test | $A_1$ | $B_1$ | $C_1$ | $D_1$ | $E_1$ | $F_1$ | $A_2$* | $B_2$* | $C_2$* | $D_2$* | $E_2$* | $F_2$* | $A_3$ | $B_3$ | $C_3$ | $D_3$ | $E_3$ | $F_3$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| K | D | 500 | 1550 | 1525 | 1500 | 1850 | 475 | 550 | 1375 | 1425 | 1500 | 1300 | 500 | 575 | 1475 | 1400 | 1625 | 1200 | 475 |
| | WB | 1750 | 5125 | 5975 | 4930 | 5375 | 1100 | 1230 | 4560 | 5312 | 5680 | 4687 | 1200 | 1370 | 5430 | 4750 | 5180 | 4250 | 1100 |
| Mg | D | 120 | 40 | 40 | 30 | 30 | 110 | 130 | 60 | 70 | 70 | 70 | 140 | 120 | 50 | 40 | 50 | 50 | 120 |
| | WB | 300 | 125 | 125 | 125 | 125 | 300 | 325 | 185 | 175 | 175 | 175 | 375 | 275 | 155 | 100 | 125 | 150 | 200 |
| Ca | D | 600 | 400 | 300 | 650 | 600 | 1700 | 800 | 550 | 550 | 600 | 600 | 850 | 800 | 350 | 400 | 400 | 600 | 400 |
| | WB | 4000 | 3250 | 3125 | 4000 | 3750 | 4125 | 4500 | 3625 | 3500 | 3750 | 3500 | 4125 | 4750 | 3000 | 3500 | 3250 | 3875 | 4000 |
| $PO_4$ | D | 400 | 300 | 300 | 300 | 100 | 400 | 300 | 200 | 400 | 300 | 100 | 300 | 400 | 300 | 300 | 300 | 100 | 400 |
| | WB | 1500 | 1250 | 1000 | 1250 | 525 | 1375 | 1275 | 1575 | 1375 | 1500 | 625 | 1750 | 1625 | 1625 | 1375 | 1625 | 600 | 1625 |
| Mn | D | 800 | 300 | 700 | 600 | 500 | 900 | 800 | 300 | 600 | 500 | 500 | 800 | 900 | 300 | 600 | 500 | 500 | 900 |
| Visual deficiency symptoms | | nil | nil | nil | N | nil | nil | nil | nil | nil | N | nil | nil | nil | nil | nil | N | nil | nil |

* $MgSO_4$ applied to all plots in Block II.

Diffusion and Waring blendor data : K, Mg, Ca, $PO_4$, Cl as $\mu$g per gm. fresh weight.
Mn as $\mu g \times 10^{-3}$ per gm. fresh weight.

usually have lower values than the NPK plants, especially in potato. Visual symptoms of the deficiency did not appear until very late in the season and even then only the habit of P deficiency was observed.

*Nitrogen.* The full chemical analysis for potato reflects the deficiency in the PK treatments.

*Manganese.* The diffusion and full chemical methods show that the levels throughout are adequate in all crops other than sugar beet, where very slight deficiency symptoms were observed early in the season, especially in the FYM treatments.

**Experiment 2.**

The chemical status of the potato (Kerr's Pink) is given in Table V. The three methods of analysis were used and comparison made with visual symptoms and yield data.

*Potassium.* Low levels of this nutrient are shown by the three methods in the NP and N only treatments. Symptoms of K deficiency were marked in the NP treatment, K and P deficiency symptoms were present in the N only treatment and late in the season K deficiency was observed in the NaCl treatments. The yield data show a significant reduction when K is omitted, as in NP and N only treatments, and yields are also lower than in NPK plots when K deficiency occurs late in the season, as in the NaCl treatments.

*Magnesium.* This nutrient is present at a deficiency level in the NP and NPK treatments where symptoms were noted. High levels were found in the NP (omit K) and NaCl treatments, probably due to the interaction with K deficiency. The Kerr's Pink and Red King varieties were more susceptible to the deficiency than Majestic and the chemical values are usually higher in the last variety. Magnesium deficiency, under these conditions, did not appear to have an appreciable effect on the reduction of yields.

*Calcium.* The chemical methods show an adequate content at the three levels, irrespective of treatment, thus showing agreement with 2—5% free lime in the surface soil. The higher level in the absence of K, shown by the chemical data, is a common phenomenon.

*Phosphorus.* The chemical values confirm the main treatments and show that P is near the deficient point in the N only and NK treatments. Symptoms of the P habit were noted but yields did not appear to be significantly lower than for NPK treatments.

*Chloride.* The diffusion and Waring blendor methods show particularly high levels in the chloride treated plants and symptoms of chloride injury were recorded at this stage. The chloride levels in Majestic are usually lower than for Kerr's Pink and Red King.

*Nitrogen.* Full chemical analysis confirms a shortage of N in the PK plots but the levels are not as low as is usual for this deficiency.

*Manganese.* The diffusion and full chemical methods show that this element is present at normal levels and that there is a tendency for values to be higher in the absence of K.

**Experiment 3.**

The chemical and visual data for potato and tomato grown in sand culture under various nutrient treatments are given in Table VI.

It is clear that the Waring blendor methods can differentiate between the treatments and that the results are in accord with those of ash analysis.

TABLE V

RELATION BETWEEN THE DIFFUSION (D) AND WARING BLENDOR (WB) TESTS, ASH ANALYSIS (AA), YIELD DATA AND VISUAL SYMPTOMS IN POTATO (KERR'S PINK). (SAMPLED 5/8/48).

| Nutrient Tested | Type of Analysis | N only 1 | N only 2 | N only 3 | NPK 1 | NPK 2 | NPK 3 | PK (omit N) 1 | PK (omit N) 2 | PK (omit N) 3 | NK (omit P) 1 | NK (omit P) 2 | NK (omit P) 3 | NP (omit K) 1 | NP (omit K) 2 | NP (omit K) 3 | FYM 1 | FYM 2 | FYM 3 | KCl 1 | KCl 3 | KCl 5 | NaCl 2 | NaCl 4 | NaCl 6 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| K | D | 250 | 180 | 150 | 1440 | 1320 | 1200 | 1500 | 1500 | 1100 | 1200 | 1500 | 1500 | 100 | 150 | 100 | 1050 | 1200 | 1000 | 1250 | 1100 | 1250 | 620 | 750 | 920 |
| | WB | 2000 | 1800 | 1500 | 10000 | 9500 | 8500 | 10250 | 9500 | 9000 | 9000 | 9750 | 10250 | 500 | 800 | 750 | 9500 | 10000 | 8000 | 10250 | 9000 | 9500 | 3750 | 3500 | 3150 |
| | AA | 1·4 | 1·07 | 0·81 | 5·13 | 5·08 | 4·62 | 4·9 | 5·42 | 4·27 | 4·04 | 5·13 | 4·63 | 0·76 | 0·73 | 0·70 | 3·91 | 4·16 | 3·77 | 4·13 | 4·20 | 4·32 | 2·10 | 2·46 | 2·20 |
| Mg | D | 120 | 110 | 100 | 40 | 30 | 40 | 20 | 20 | 10 | 50 | 60 | 60 | 120 | 130 | 130 | 60 | 50 | 50 | 50 | 50 | 50 | 70 | 80 | 70 |
| | WB | 500 | 525 | 450 | 100 | 125 | 100 | 75 | 50 | 50 | 225 | 250 | 250 | 550 | 600 | 650 | 250 | 225 | 225 | 275 | 250 | 250 | 350 | 450 | 450 |
| | AA | 1·22 | 1·40 | 1·14 | 0·36 | 0·35 | 0·34 | 0·20 | 0·30 | 0·26 | 0·46 | 0·52 | 0·43 | 1·47 | 1·36 | 1·50 | 0·50 | 0·42 | 0·45 | 0·44 | 0·44 | 0·44 | 0·68 | 0·78 | 0·71 |
| Ca | D | 2000 | 1750 | 1500 | 1000 | 1250 | 1500 | 1750 | 1500 | 1500 | 1750 | 1500 | 1500 | 2000 | 2000 | 1750 | 1500 | 1500 | 1500 | 1250 | 1500 | 1500 | 2250 | 2150 | 2500 |
| | WB | 4500 | 4990 | 4350 | 3120 | 3100 | 3900 | 3490 | 3250 | 3700 | 3300 | 3250 | 3250 | 4850 | 4450 | 4650 | 3900 | 3870 | 3750 | 3200 | 3800 | 3500 | 3300 | 3500 | 3500 |
| | AA | 5·92 | 5·81 | 5·49 | 3·2 | 3·2 | 3·43 | 4·0 | 3·7 | 3·65 | 3·7 | 3·6 | 3·50 | 5·9 | 5·6 | | 3·5 | 3·8 | 3·46 | 3·15 | 3·87 | 3·45 | 5·25 | 5·43 | 5·73 |
| P | D | 100 | 100 | 150 | 300 | 400 | 350 | 400 | 450 | 400 | 100 | 150 | 100 | 350 | 300 | 500 | 250 | 300 | 250 | 250 | 300 | 250 | 250 | 200 | 200 |
| | WB | 400 | 350 | 350 | 750 | 900 | 850 | 700 | 750 | 700 | 300 | 350 | 350 | 750 | 700 | 730 | 650 | 700 | 600 | 500 | 550 | 500 | 600 | 550 | 500 |
| | AA | 0·46 | 0·48 | 0·34 | 0·66 | 0·80 | 0·76 | 0·74 | 0·75 | 0·72 | 0·36 | 0·40 | 0·31 | 0·82 | 0·75 | | 0·67 | 0·77 | 0·66 | 0·55 | 0·52 | 0·47 | 0·58 | 0·54 | 0·53 |
| Cl | D | 100 | 100 | 100 | 100 | 200 | 100 | 100 | 100 | 200 | 100 | 100 | 100 | 100 | 100 | 100 | 200 | 100 | 100 | 600 | 600 | 700 | 600 | 700 | 700 |
| | WB | 1000 | 750 | 750 | 1000 | 750 | 1000 | 750 | 500 | 1000 | 1000 | 750 | 1000 | 500 | 500 | 500 | 1000 | 750 | 1000 | 7500 | 8000 | 7500 | 9000 | 8000 | 9000 |
| N | AA | 5·3 | 5·2 | 5·5 | 4·6 | 4·2 | 4·3 | 2·1 | 2·2 | 2·0 | 3·8 | 4·8 | 4·6 | 5·3 | 5·6 | 5·0 | 3·0 | 3·1 | 3·2 | 4·0 | 3·9 | 4·1 | 3·7 | 3·5 | 4·0 |
| Mn | D | 500 | 600 | 700 | 700 | 800 | 700 | 400 | 500 | 500 | 800 | 800 | 800 | 900 | 900 | 1000 | 200 | 200 | 300 | 800 | 800 | 800 | 800 | 800 | 800 |
| | AA | 51 | 60 | 99 | 90 | 85 | 72 | 50 | 55 | 52 | 80 | 75 | 80 | 100 | 110 | 100 | 30 | 25 | 35 | 85 | 90 | 90 | 75 | 80 | 70 |
| Total Yields Tons per acre. | | 6·16 | | | 15·05 | | | 6·97 | | | 11·41 | | | 6·24 | | | 13·59 | | | 17·01 | | | 15 | | |
| Visual symptoms + toxicity — deficiency | | —K | | | —Mg | | | —N —Mg | | | —P (habit only) | | | —K | | | Nil | | | —Mg (trace) | | | —K +Cl | | |

Diffusion and Waring blendor data : K, Mg, $PO_4$, Cl as $\mu$g per gm. fresh weight.
Mn as $\mu g \times 10^{-3}$ per gm. fresh weight.
Ash analysis data : % $K_2O$, MgO, CaO, $P_2O_5$ and N in dry matter.
Mn as $\mu$g per gm. dry matter.

## TABLE VI.

Relation Between Waring Blendor (WB) Test, Ash Analysis (AA) and Visual Symptoms in Potato and Tomato Grown in Sand Culture. (Sampled 30/8/48).

| Nutrient tested | Type of test | POTATO | | | | | TOMATO | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Complete nutrient | K omitted | Mg omitted | Ca omitted | P omitted | Complete nutrient | K omitted | Mg omitted | Ca omitted | P omitted |
| K | WB | 9630 | 750 | 9850 | 12120 | 12875 | 7640 | 500 | 9630 | 11500 | 7900 |
| | AA | 5·63 | 1·12 | 5·72 | 6·39 | 5·38 | 4·78 | 0·78 | 4·98 | 6·13 | 4·69 |
| Mg | WB | 525 | 500 | 50 | 475 | 425 | 275 | 350 | 25 | 350 | 250 |
| | AA | 1·18 | 1·12 | 0·28 | 1·19 | 0·88 | 0·95 | 1·26 | 0·07 | 1·16 | 0·85 |
| Ca | WB | 4725 | 6500 | 4625 | 750 | 5120 | 4650 | 6250 | 4720 | 1000 | 4250 |
| | AA | 4·60 | 6·80 | 4·73 | 1·13 | 5·21 | 5·36 | 6·95 | 4·60 | 1·61 | 4·89 |
| P | WB | 750 | 825 | 650 | 900 | 75 | 850 | 900 | 800 | 950 | 75 |
| | AA | 0·78 | 0·80 | 0·63 | 0·85 | 0·29 | 0·76 | 0·86 | 0·75 | 0·85 | 0·18 |
| Visual deficiency symptoms | | Nil | K | Mg | Ca | P | Nil | K | Mg | Ca | P |

Waring blendor data : K, Mg, Ca, $PO_4$, Cl as $\mu g$ per gm. fresh weight.

Ash analysis data : % $K_2O$, MgO, CaO, $P_2O_5$ and N in dry matter.

TABLE VII.

Relation Between Visual Symptoms, Full Chemical Analysis, Waring Blendor and Diffusion Methods of Determining the K, Mg, Ca and P Status of Lane's Prince Albert.

| Plot | Manurial Treatment | Visual Deficiency Symptoms | Chemical Data on Leaves | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | as % Dry Matter | | | | Diffusion (D) and Waring Blendor (WB) data as μg per gm. fresh weight | | | | |
| | | | CaO | MgO | $K_2O$ | $P_2O_5$ | Ca | | Mg | K | $PO_4$ |
| A1 | Nil | Nil | 2·03 | 0·27 | 1·66 | 0·31 | D<br>WB | 1000<br>2750 | 30<br>90 | 800<br>4000 | 200<br>600 |
| A2 (a) | Nil | K (Trace) | 3·30 | 0·45 | 1·34 | 0·44 | D<br>WB | 1250<br>3000 | 50<br>150 | 500<br>1500 | 300<br>650 |
| A2 (b) | 2% $K_2SO_4$ foliage spray | Nil | 1·38 | 0·27 | 2·63 | 0·32 | D<br>WB | 500<br>1900 | 30<br>100 | 1500<br>6280 | 200<br>550 |
| B1 | Complete Inorganic | Mg | 1·85 | 0·15 | 3·28 | 0·37 | D<br>WB | 850<br>2250 | 20<br>60 | 1750<br>5270 | 200<br>575 |
| B2 (a) | 2% $MgSO_4$ foliage spray | Mg | 2·26 | 0·29 | 2·04 | 0·62 | D<br>WB | 1000<br>3000 | 30<br>75 | 1000<br>2500 | 400<br>850 |
| B2 (b) | | Nil | 1·03 | 1·30 | 1·73 | 0·33 | D<br>WB | 500<br>1750 | 150<br>550 | 900<br>3250 | 150<br>400 |
| C1 | FYM | Mg | 1·89 | 0·26 | 2·29 | 0·32 | D<br>WB | 800<br>2500 | 30<br>80 | 1100<br>5900 | 150<br>450 |
| C2 | ,, | Nil | 2·50 | 0·34 | 2·35 | 0·67 | D<br>WB | 1250<br>3750 | 40<br>140 | 1250<br>6820 | 400<br>800 |
| D1 | PK (omit N) | Mg | 2·29 | 0·29 | 3·56 | 0·41 | D<br>WB | 1050<br>3250 | 30<br>80 | 2000<br>8500 | 300<br>675 |
| D2 (a) | PK (omit N) | Mg | 2·98 | 0·17 | 1·97 | 0·42 | D<br>WB | 1250<br>3000 | 10<br>30 | 1000<br>4120 | 250<br>600 |
| D2 (b) | 1% $MgSO_4$ foliage spray | Nil | 1·03 | 0·94 | 1·77 | 0·34 | D<br>WB | 450<br>1750 | 100<br>350 | 1000<br>4250 | 200<br>600 |
| D2 (c) | 2% $MgSO_4$ foliage spray | Nil | 0·97 | 1·31 | 1·48 | 0·34 | D<br>WB | 400<br>1500 | 170<br>650 | 700<br>2120 | 200<br>600 |
| E1 (a) | NK (omit P) | Mg | 2·15 | 0·23 | 2·51 | 0·39 | D<br>WB | 800<br>2500 | 30<br>80 | 1500<br>5900 | 250<br>675 |
| E1 (b) | 2% $MgSO_4$ foliage spray | Nil | 1·58 | 0·57 | 3·04 | 0·43 | D<br>WB | 700<br>2000 | 90<br>325 | 1750<br>6280 | 300<br>750 |
| E2 (c) | | Mg | 2·39 | 0·31 | 1·59 | 0·33 | D<br>WB | 1150<br>3000 | 40<br>150 | 900<br>2900 | 200<br>550 |
| F1 (a) | NP (omit K) | Nil | 2·90 | 0·39 | 1·72 | 0·43 | D<br>WB | 1250<br>3000 | 40<br>120 | 800<br>2160 | 250<br>700 |
| F1 (b | 2% $K_2SO_4$ foliage spray | Nil | 1·08 | 0·27 | 2·27 | 0·38 | D<br>WB | 500<br>1750 | 30<br>90 | 1500<br>5800 | 250<br>650 |
| F2 | | K | 1·28 | 0·29 | 1·35 | 0·44 | D<br>WB | 700<br>1750 | 30<br>80 | 500<br>1320 | 300<br>750 |

Rates of application per acre :
FYM—10 tons ; nitrate of soda—2 cwt.; superphosphate—3 cwt.; muriate of potash—1 cwt.

**Experiment 4.**

The relation between chemical results, leaf symptoms and manurial treatment for apple (Lane's Prince Albert) are given in Table VII. The chemical data roughly parallel one another and show the major differences in treatment. Thus in the absence of K, as in the nil and NP plots, the values for the nutrient are lower and the deficiency was observed in $A_2$ (a) and $F_2$. The efficacy of foliage sprays of 2% potassium sulphate in correcting the deficiency is indicated by higher values for the nutrient in these treatments, although the results may be too high, due to the accumulation of spray residue on leaf surfaces.

Magnesium deficiency is shown in plots which were given PK and NPK treatments. Values are usually lower than for those receiving a foliage spray

of magnesium sulphate either at the 1% or 2% rates. The low level of Mg in the omit K trees is noteworthy as, contrary to the potato under similar treatment when K deficiency results in an increase in Mg value, K and Mg are both low in the tissues.

The rapid tissue test methods and ash analysis show a normal Ca level throughout.

The phosphorus values appear to be adequate.

## CENTRE B.

Details of the Waring blendor, ash analysis and visual data for potato (Majestic) appear in Table VIII. The main points are as follows :—

*Potassium.* In the unlimed group low levels for K are shown in the omit K plots by the two chemical methods and the K deficiency habit was recorded in the crop. The chemical results show that the K treatments are better differentiated in the limed group.

*Magnesium.* As in previous years, the deficiency appeared in the PK and NPK treatments in the unlimed plots and in all treatments receiving K in the limed group. The Waring blendor and ash analysis methods confirm these effects.

*Calcium.* The effect of liming on the Ca level in the plants is well shown by both methods and deficiency effects were noted in some of the unlimed plots.

*Phosphorus.* The presence of the deficiency in the NK plots and K-lime treatment is confirmed by the blendor and ash analysis data. Low values are also recorded in the nil plots but under these conditions other symptoms may mask those of P deficiency.

*Manganese.* There are significant differences between the limed and non-limed groups, and toxicity symptoms of the element were recorded in some treatments in the absence of lime. Although the Waring blendor data show significant differences in Mn values for the limed and unlimed group, these should be regarded as relative data, as the carbon in the blendor can adsorb approximately 10% of the Mn in the tissue extract.

## DISCUSSION.

The diffusion method for the extraction of mineral nutrients from fresh plant material has been shown to be of value in the diagnosis of mineral disorders in crop plants (9). In this paper details of a Waring blendor method, using the acetate buffer solution, are given. This procedure is quicker in operation than the original diffusion method. Comparisons have been made between the two rapid methods and the results of ash analysis, using several crops growing under widely different soil conditions with regard to mineral status. The chemical data for the three chemical methods are at different levels but in most instances roughly parallel one another, so that they give a similar assessment of the mineral status of the crops. The Waring blendor method is less precise than the more elaborate one of ash analysis but major differences between treatments are shown and a diagnosis of mineral status of the crop is possible using the quick method. The Waring blendor procedure is quicker in operation than the diffusion method and the extraction of the nutrients is less tedious and more standardised.

Comparisons between the visual diagnosis method and the chemical results enable the fixing of threshold of deficiency levels which coincide with

## TABLE VIII.

Relation Between Waring Blendor (WB) Test, Ash Analysis (AA) and Visual Symptoms in Potato (Majestic).
(Sampled 20.8.48).

| Nutrient tested | Method of test | Unlimed | | | | | | | | Limed at 9 tons per acre. | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Nil | NPK | PK | NK | K | NP | P | Nil | Nil | NPK | PK | NK | K | NP | P | Nil |
| K | WB | 1450 | 5950 | 6000 | 6500 | 5050 | 1900 | 1550 | 1250 | 850 | 12800 | 14000 | 9400 | 11450 | 1050 | 850 | 1650 |
| | AA | 1·48 | 3·85 | 3·73 | 3·12 | 3·40 | 1·60 | 1·57 | 1·60 | 1·28 | 7·20 | 7·18 | 5·33 | 5·60 | 1·39 | 1·19 | 2·13 |
| Mg | WB | 275 | 100 | 120 | 275 | 150 | 275 | 150 | 250 | 275 | 120 | 160 | 75 | 75 | 225 | 245 | 250 |
| | AA | 0·50 | 0·35 | 0·38 | 0·46 | 0·32 | 0·49 | 0·34 | 0·42 | 0·49 | 0·36 | 0·41 | 0·33 | 0·24 | 0·46 | 0·45 | 0·39 |
| Ca | WB | 500 | 350 | 400 | 625 | 325 | 475 | 625 | 550 | 5600 | 3250 | 3500 | 3750 | 3725 | 2500 | 4000 | 4700 |
| | AA | 1·01 | 0·61 | 0·68 | 1·01 | 0·59 | 0·79 | 1·10 | 1·07 | 5·69 | 4·82 | 4·66 | 4·60 | 4·48 | 4·20 | 4·81 | 5·40 |
| P | WB | 250 | 800 | 900 | 250 | 300 | 700 | 800 | 300 | 350 | 650 | 750 | 260 | 250 | 850 | 850 | 300 |
| | AA | 0·33 | 1·18 | 1·17 | 0·38 | 0·46 | 0·74 | 0·82 | 0·40 | 0·47 | 0·64 | 0·81 | 0·39 | 0·30 | 1·33 | 0·89 | 0·38 |
| Mn | WB | 375 | 320 | 300 | 320 | 250 | 360 | 390 | 300 | 25 | 20 | 25 | 10 | 10 | 25 | 25 | 20 |
| | AA | 860 | 704 | 628 | 713 | 615 | 800 | 880 | 725 | 133 | 127 | 139 | 123 | 132 | 120 | 126 | 142 |
| Visual symptoms | | —Ca | —Ca | —Ca | —Ca | —Ca | —K (habit) +Mn | —K (habit) +Mn | —Ca | —K | —Mg | —Mg | —Mg | —Mg | —K | —K | —K |
| — deficiency | | —K (habit) | —Mg | —Mg | —P (habit) | —Mg | | | —K (habit) | | | | —P (habit) | —P | | | |
| + toxicity | | +Mn | +Mn | +Mn | +Mn | | | | +Mn | | | | | | | | |

Diffusion and Waring blendor data : K, Mg, Ca, $PO_4$, Mn as $\mu$g per gm. fresh weight.

Ash Analysis data : % $K_2O$, MgO, CaO, $P_2O_5$, in dry matter.
Mn as $\mu$g per gm. in dry matter.

the onset of symptoms. This has already been done for the diffusion and ash analysis methods (3, 9). It is difficult to determine similar levels for the Waring blendor extracts on one season's work ; nevertheless tentative values can be suggested. Thus for K in potato, values between 1,000 to 2,000 $\mu$g per gm. fresh weight may be critical, as at Centre A, Experiments 1 and 2, and Centre B. Deficiency symptoms appeared at these levels and moreover at the first two centres were associated with reduction in yields. These results can be compared with those obtained by other workers. Carolus, using a mortar grinding method for extraction of nutrients (1, 2) suggests that approximately 2,000 and 4,000 p.p.m. K at the beginning and end of season respectively may be regarded as a minimum level, below which yields of tubers are depressed. Lorenz (7) found that soluble K in his potato samples were over 8,000 p.p.m. in all treatments and that K manuring had no effect on the yields. Hill and Cannon (6) suggest that when levels are below 3,500 p.p.m., yields are depressed. It is clear from a comparison of the data given in this paper and those of other investigators mentioned, that critical levels for onset of K deficiency symptoms are below those associated with reduction in crop yields. Threshold levels for K, associated with deficiency symptoms in cauliflower, broad bean, linseed and apple are usually below 2,000 $\mu$g per gm.

For Mg less than 200 $\mu$g per gm. for potato and 150 $\mu$g per gm. for apple and cauliflower may be suggested as threshold levels. Hill and Cannon (6) found 365 p.p.m. Mg to be adequate for optimum yields in potato, although Carolus suggested 275 p.p.m. as minimum level, below which yields are adversely affected.

Values below 1,000 $\mu$g per gm. Ca may be associated with the deficiency in potato, and values below 100 $\mu$g per gm. $PO_4$ for phosphorus deficiency. Minimum P levels for potato, given by Carolus (1, 2), Lorenz (7) and Hill (6) are 60 to 150, less than 23 and less than 42 $\mu$g P respectively.

For Mn and Cl toxicity, symptoms usually appear when Waring blendor values are over 200 and 5,000 $\mu$g per gm. respectively.

Several investigators have attempted to correlate the nutrient status of crops with yields (3). Moreover, the critical values quoted for the nutrients are usually associated with reduction in yields. In this investigation, whenever possible, yield data are given. Thus at Centre A, potatoes in the NP (omit K) treatment had significantly lower K status and total yield values than those in the NPK treatment.

Although preliminary data show the value of the Waring blendor for the extraction of nutrients, further work is in progress to compare the relative merits of other homogenisers, including the N.I.R.D. macerator. Moreover the use of other extracting solutions will be tried to determine which is best correlated with crop performance.

## SUMMARY.

1. A Waring blendor method is described which has been used for the determination of the mineral status of K, Mg, Ca, $PO_4$, Cl and Mn in a variety of crops, including potato (3 varieties), cauliflower, broad bean, flax, oat, barley and apple, grown at various centres.

2. The method showed the main differences between the manurial treatments at the various centres.

3. Comparisons made between Waring blendor, diffusion and ash analysis data showed that the three methods gave values at different levels but that these were roughly in parallel.

4. The Waring blendor method is probably quicker in operation than the diffusion method and is also a more standardised procedure. It cannot, however, be used as a field method.

5. Correlations between the blendor data and those of the visual method of diagnosis are usually close. Tentative threshold levels which coincide with development of symptoms, based on one season's work, are given.

6. Yield results for potato are significantly correlated with the K status of the plants as determined by the three chemical methods.

7. It is suggested that further work is necessary to determine the best type of maceration of tissues for the extraction of mineral nutrients and to relate the data to crop performance.

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# RADIOACTIVE ISOTOPES—AN INTRODUCTION TO THEIR PROPERTIES AND APPLICATIONS TO PLANT NUTRITIONAL STUDIES

By W. D. E. THOMAS

The use of radioactive isotopes as ' tracers ' in the solution of biochemical and chemical problems has received a tremendous impetus from the existence of the Atomic Energy Research Establishment at Harwell, Didcot. Radioactive isotopes are produced there and are now available to research institutes and universities; interest in the technique of using radioactive tracers has therefore become widespread. Another factor contributing to the increasing use of radioactive isotopes is the availability of the necessary electronic equipment which has simplified considerably the quantitative assaying of radioactivity. In this introduction to the subject, it is proposed to refer to the historical development of the knowledge of atomic structure, to outline briefly the nature of radioactive isotopes, and to illustrate the principles of radioactive tracer work by a simple example. Such an approach, it is hoped, will indicate the potentialities of the technique and also draw attention to the factors which tend to limit its usefulness.

If we entered a science class room about 60 years ago and heard a lecture on the structure of matter, we should probably be impressed by the apparent finality of the state of knowledge, and might well wonder what the physicists of the future would find to occupy their time. We should learn that a piece of matter, such as copper, was composed of a very large number of discrete particles called atoms. These atoms, all of them much too small to be seen, were then said to be chemically indivisible and completely unchangeable. All the atoms of one element were identical in weight, and differed in weight from atoms of all other elements. This concept received considerable support from the Periodic Table of the Elements in which it was shown that when the known elements were arranged in order of increasing atomic weights, a striking periodicity of properties was displayed.

These somewhat superficial views of the structure of matter were rather rudely shaken by observations made at the close of the last century. In 1896 Becquerel happened to leave some new photographic plates in a drawer in his laboratory. When he came to use and then to develop them some time later, he found that they had been completely fogged, but instead of discarding them, Becquerel sought the reason for the fogging, and eventually showed that it was due to the action of uranium salts, which happened to have been left in the drawer at the same time as the photographic plates. This was a remarkable conclusion—it indicated that the uranium salts had been giving out rays, which had penetrated the thick paper around the photographic plates, and fogged the emulsion, just as light would have done. Madame Curie amongst others tried to resolve this mystery. She showed that of the known elements only thorium and uranium salts had this power. She then examined the pitchblende ore from which these elements were extracted, and to her amazement found that the parent mineral gave out far more intense radiations than the two elements which had been separated from it. This could be explained only by the presence in pitchblende ore of some new element of greater radioactivity. After a laborious extraction process, Madame Curie isolated two new elements,

one of them radium, both of which were found to emit some very penetrating rays.

These were thrilling days in the development of knowledge concerning atomic structure, and it was not long before Rutherford and others found why a substance like radium, which was a definite element, could throw out these penetrating rays, and go on doing so apparently indefinitely. They postulated that the radium atoms were fundamentally unstable, that a small proportion of them was continually exploding, and that such explosions, or disintegrations as they were called, were accompanied by the emission of energy in the form of rays. As each radium atom exploded it changed into an atom of another element, which in turn was also radioactive. The final product in this chain of events was the stable element lead, though it takes millions of years for an appreciable amount of lead to be formed this way. It was shown that the activity of radium was the same whether it was present as an element or in the form of a compound such as radium chloride ; nor was it affected by extremes of temperature or any known chemical or physical treatments.

Rutherford found that the rays emitted by all radioactive elements were of at least three kinds—which he empirically called $\alpha$, $\beta$ and $\gamma$.

$\alpha$ radiation he showed to be due to positively charged particles identical with the nuclei of helium atoms.

$\beta$ radiation were shown to consist of a stream of electrons. The weight of each electron is about 1/1846 that of the hydrogen atom, and it bears a single negative charge. It is this type of radiation which is encountered most often in radioactive tracer work.

$\gamma$ rays were electromagnetic waves, differing only from wireless waves and visible light in having a very short wavelength, shorter even than the hardest X-rays.

**Atom Structure.**

All this new information complicated the ideas about atomic structure considerably, and it may be useful at this stage to give some indication of the more modern view of atom structure. The popular physical picture of the atom is still that first given by Bohr and Rutherford, whose nuclear atom theory, developed about 1912, represented atoms as having a common pattern somewhat analogous to our solar system. Each atom consists of a nucleus with a complement of electrons encircling it just as the planets do the sun. The atom as a whole is minute, about one hundred millionth of a cm. in diameter, but the diameter of the nucleus is very much smaller than this, actually only about 1/10,000 of the diameter of the atom. It is from this very small nucleus however that the $\alpha$, $\beta$ and $\gamma$ radiations emanate.

The principal constituents of every atom nucleus are protons and neutrons, usually in about equal numbers—hydrogen is an exception, its nucleus consists of one proton. For reference the relevant properties of these two constituents, and of the satellite electrons, are summarised below :—

Proton— Nucleus of hydrogen atom. Carries single positive charge
Mass = 1 (1·00758, Oxygen = 16·0000)
Constituent of nucleus of every atom.

Neutron—Carries zero charge. Mass = 1 (1·00897 ; Oxygen = 16·0000)
Constituent of nucleus of every atom except hydrogen.

Electron—Single negative charge. Mass = 1/1845 proton.
Constituent of every atom.

Since the protons each carry a single positive charge and since the atom as a whole is neutral, the negative charges required for balance are provided by electrons, equal in number to the protons, but moving in orbits around the nucleus. The balance of mass of the atom is due to the presence of neutrons in the nucleus—in fact practically the whole of the atom weight is due to the protons and neutrons in the nucleus since the mass of the electrons is so very small in comparison.

(It should be emphasised that this concept is a simplified one, and that it would be more in keeping with modern conceptions of wave mechanics to consider the electrons as a cloud having wave functions, and to regard the electron orbits merely as zones of probability. For our purpose, however, the simplified picture of the atom consisting of a positively charged nucleus containing about an equal number of protons and neutrons, with negatively charged electrons, equal in number to the nuclear protons, coursing round in their orbits, in a very ' empty ' atom, is sufficient.)

To take an example—phosphorus. This has an atomic weight of 31 (relative to Oxygen = 16), with a nucleus consisting of 15 protons and 16 neutrons. To balance the positive charge on the nucleus there must be 15 electrons in the space surrounding the nucleus. Sulphur, the element next to phosphorus when they are arranged in order of increasing atomic weights, has 16 protons and 16 neutrons in its nucleus, and therefore 16 electrons spinning around the nucleus.

It is now accepted that the chemical properties of the elements are functions of the number of electrons in their individual atoms. Thus phosphorus is different from sulphur because the number of protons in the nuclei of the atoms of the two elements is different, resulting in the sulphur and phosphorus atoms having a different number of electrons surrounding their nuclei. The number of protons in the nucleus, which thus controls the chemical identity of the atom, is called the ATOMIC NUMBER and is of far more fundamental importance than the atomic weight.

Since the mass of the atom is practically equal to the mass of the nucleus (an electron weighing only 1/1845 of the proton weight), and since the masses of the neutron and proton are practically unity on our scale of weights, it would be anticipated that the atomic weights of the elements should be whole numbers, or nearly so. This was true of many elements, the atomic weight of phosphorus being 30·98, that of helium 4·00, beryllium 9·02, and carbon 12·01, but exceptions were known which did not conform to this scheme. The classic example was chlorine, the accurately determined atomic weight for which was 35·46.

**Isotopes.**

This anomaly was resolved as a result of yet another revolutionary suggestion, made by Soddy in 1913. He was concerned only with the naturally radioactive series of elements, and showed that two forms of the same element could exist, each possessing different atom weights. The term ' isotope ' was proposed by him for two or more such species of the same element in which the atom weights were different. The non-radioactive elements were investigated from 1919 onwards by Aston, who by means of his mass spectrograph showed that most elements consisted of two or more stable isotopes (phosphorus was one of the exceptions—it had no stable isotope). In particular, chlorine consists of atoms, some of atomic weight 35·0 and others 37·0, occurring in the same relative proportions in chlorine obtained from

any part of the world. Unless therefore some very specialised technique is used to separate the isotopes from each other, the atomic weight of the 'mixture' (i.e. the conventional atomic weight) will be the same whatever the source of the element. That is true for most elements, lead being a notable exception. In certain minerals in which it has degraded from thorium its atomic weight is 208, but lead resulting from the disintegration of uranium 238 has an atomic weight of 206, whilst if resulting from uranium 235 the atomic weight is 207. The atomic weight of lead obtained from the mineral galena is 207·21. This remarkable dependence of the atomic weight of lead on its radioactive source has been used as a basis for estimating the age of the earth.

It is important to understand the meaning of isotopes quite clearly. Two or more isotopes of the same element differ in mass or weight, but they possess the same atomic number. In other words, the atoms of such isotopes possess the same number of protons combined with a different number of neutrons. Thus the two chlorine isotopes both have atoms with nuclei containing 17 protons combined with 18 neutrons and 20 neutrons respectively. Since the number of protons is the same in both species the number of planetary electrons will be the same and hence they will have identical chemical properties. So far nothing has been stated regarding the number of neutrons which can co-exist indefinitely with a fixed number of protons in the same atom. In the chlorine atoms we have seen that 17 protons can remain associated indefinitely in a stable nucleus with 18 or 20 neutrons. One might wonder what restriction there is, and why there are not more isotopes. However, any definite number of protons can exist with only a fixed number of neutrons, usually about an equal number. If, however, we vary this ratio a little, for example by introducing one more neutron, what are the possibilities? It might be that the extra neutron would be incorporated in the nucleus and a perfectly stable atom produced—an isotope of the original element. At the other extreme it is possible that such a liaison would be too unstable to exist even momentarily, and the impact of the neutron on the nucleus would be simultaneously followed by its ejection. In between these two extremes, there is a possibility that the intruding neutron would result in the formation of a nucleus which is unstable, too unstable to exist indefinitely, but stable enough to hold together for some time; sooner or later, however, the system will adjust itself to a more lasting arrangement. The process of adding neutrons, perhaps the commonest way of making radio-active isotopes, is exactly what goes on in the atomic pile at Harwell.

**Radioactive Phosphorus.**

To take a concrete example. Suppose we put red phosphorus in the pile. This will be bombarded by millions of neutrons every second, and occasionally an invading neutron will happen to hit the actual nucleus of one of the phosphorus atoms—this will be quite a rare event because the nuclei are so small. However, each time a neutron does happen to hit the nucleus of a phosphorus atom, it remains. The atoms in the phosphorus which went into the pile each had 16 neutrons and 15 protons in their nuclei—giving an atomic weight of 31—symbolised as $P^{31}_{15}$. If a neutron enters an atom of $P^{31}$ the new atom would still have 15 protons but 17 neutrons, giving an atomic weight of 32. Since the number of protons is the same, 15, the number of planetary electrons is the same and the new atom will still be phosphorus—a radioactive isotope of phosphorus; however, because of the extra neutron in the nucleus

it will be under a strain and sooner or later will get rid of this strain. One way would be for each radioactive atom in the phosphorus to eject the invading neutron, but what actually does occur in this case is that an electron is expelled from one of the neutrons in the nucleus of the $P^{32}$ atoms. This transforms one neutron into a proton in each disintegrating nucleus, and the residual atom with 16 protons and 16 neutrons in its nucleus is what we call sulphur, the atoms of which are of course quite stable. Disintegrations of radioactive phosphorus will thus result in formations of extremely small amounts of sulphur.

$$P^{32}_{15} \longrightarrow S^{32}_{16} + \beta$$

It is of interest to know something about the number of radioactive atoms in an irradiated sample of material. If about a gram of phosphorus was irradiated for a week in the Harwell Atomic Pile it would have become radioactive because a small proportion of the $P^{31}$ atoms had become $P^{32}$ atoms. To give an estimate of the efficiency of neutron hits on the nuclei of the $P^{31}$ atoms in the Harwell Pile, it can be said that during such an irradiation only one nucleus in about a thousand million will have been hit, so that the 1 gram of ordinary phosphorus put into the pile will contain about one thousand millionth gram of the radioactive phosphorus isotope $P^{32}$, after irradiation. The $\beta$ particles emitted by the disintegration of such a small weight of $P^{32}$ would nevertheless be more than sufficient to require very careful precautions in its handling.

Radioactive isotopes may be created by a great variety of reactions, but the pattern is the same. A particle such as a neutron enters the nucleus ; the compound nucleus so formed is unstable, and sooner or later ejects another particle. This process of disintegration does not take place instantaneously. Some of the atoms decompose immediately, others may take minutes or years or even thousands of years before they break down. In fact, the disintegation is a chance phenomenon, and the number disintegrating per unit time is proportional to the number of radioactive atoms present. It is found that after a definite time, which is a characteristic of each radioactive isotope, half of the total number of radioactive atoms present at the beginning of that time will have disintegrated. This time interval is called the '*half life*' for the radioactive substance, and is a very important property in determining the usefulness of an isotope for biological use. The ' half life ' for $P^{32}$ is 14 days, whilst for Carbon$^{14}$ it is about 5,000 years. It should be emphasised that the rate of disintegration, whether it be fast or slow, in any radioactive element is completely independent both of the chemical form in which the element is combined and the external physical conditions.

**Measurement of radioactivity.**

The next logical step is to discuss the quantitative measurement of radioactivity. In fact the whole technique of using radioactive isotopes depends on there being available a means of assessing, qualitatively or quantitatively, the radioactivity in any specimen. It is said that a substance is radioactive because it emits radiation, and as far as we are concerned now we can confine ourselves to those radioactive bodies which emit electrons or $\beta$ particles. This type of radiation, which occurs with $P^{32}$, is the simplest of all to deal with. Our problem in quantitative assaying of radioactivity resolves itself to this. Suppose we have several samples each known to contain $P^{32}$ and we wish

to express the ratio of their activities quantitatively. We know that radioactivity depends on the disintegrations of $P^{32}$ atoms and we also know that each disintegration results in the shooting out to space of one electron. So if there are 5,000 atoms disintegrating per minute 5,000 electrons will be shot out from the specimen per minute. From this the obvious way to assess the activity of the sample would be to count all of these electrons and calculate an average per minute. This in fact is what is done by the Geiger Muller counter—the only point being that instead of counting all the electrons being

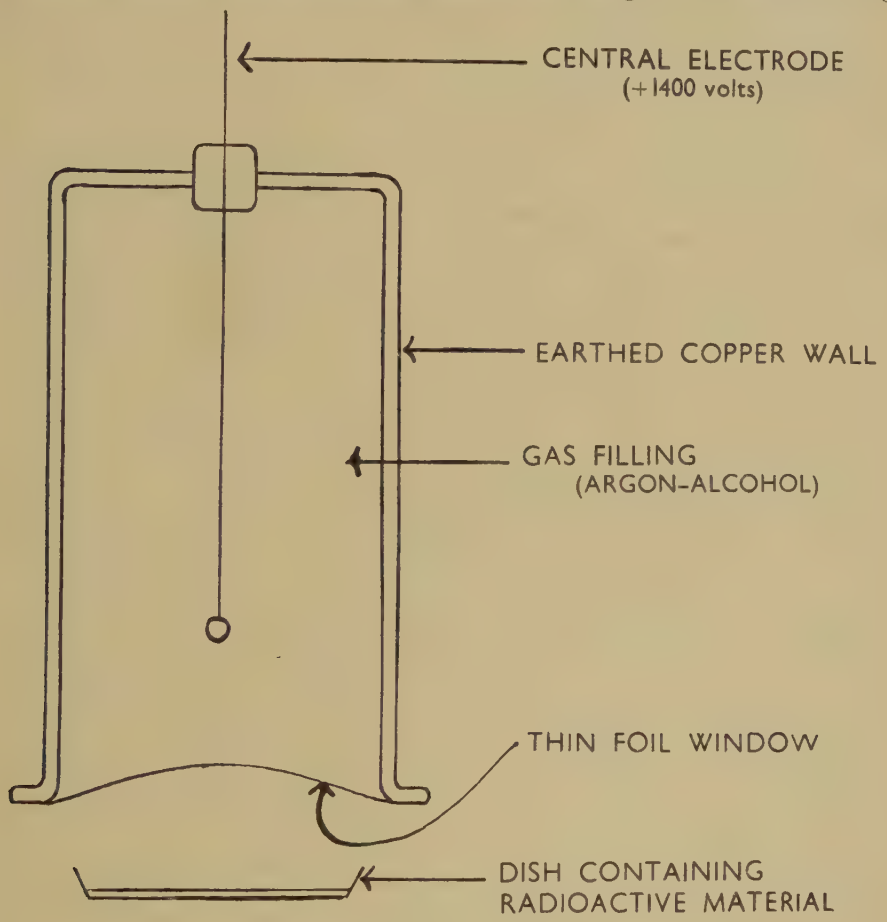


*Fig.* 1. Section of Counter Tube.

given off—they will of course be given off in all directions—we count a constant proportion of them. If this proportion is kept the same throughout our determinations then, unless we are interested in absolute data—which is very seldom the case—all the results obtained will be mutually consistent and comparable. This counting operation is, however, a little complicated because the electrons may be given off at anything from 30 to 15,000 per minute—the range of activity normally encountered in tracer work. The Geiger Muller counter tube (*Fig.* 1) consists of a cylindrical tube, usually of copper, through one end of which is inserted a tungsten wire, which acts as the positively charged anode. The other end of the tube is closed by a thin film of copper

or duralumin. This is deliberately made thin to facilitate the entry of the $\beta$ particles through it. The gas in such a counter is a mixture of argon and ethyl alcohol at a pressure of about a tenth of an atmosphere. The central wire or anode is maintained at a positive potential which is usually of the order of 1200—1400 volts.

Each $\beta$ particle entering through the thin end window sets up a sequence of events which results in the potential on the central wire fluctuating slightly. What happens is that as the $\beta$ particle flies through the counter tube, it impacts atoms of the gas, and before coming to rest, it knocks off an electron from some of the gaseous atoms or molecules it encounters. As soon as these secondary electrons are formed, and they may be very few in number, they are attracted towards the positively charged central wire. They accelerate as they get nearer the wire due to the increasing field strength, until when they are within a very short distance of the central wire, about 0·5 mm., they have acquired sufficient kinetic energy to induce more ionisation, and the electrons so produced can now acquire enough energy in one free path to knock off an electron from the next atom they hit. In other words there is a chain type of reaction set up in which, as a result of the entry of one $\beta$ particle into the counter, an avalanche of electrons bombards the central wire. This avalanche is terminated by the build up of a positive space charge behind the electrons. This positive space charge is due to the residual positively charged atoms or molecules, each minus an electron, and the counter will not function until these charged particles have reached the outer cathode wall and their charge has been neutralised. Thus provided the $\beta$ particles do not enter the counter at rates exceeding about 15,000 per minute, a small negative pulse or fluctuation is given to the potential on the central electrode for each $\beta$ particle entering the tube. Counting the number of $\beta$ particles which have entered the counter thus resolves itself into counting the number of pulses given to the central wire. This is done by the electronic equipment, and what happens, at least in principle, is that each little pulse is amplified and then operates a mechanical recorder—which resembles a motor car mileage meter. There is one addition common to most counters. These mechanical recorders cannot operate at 5,000 or 10,000 a minute, so a device called a scaler is usually incorporated between the pulse amplification stage and the recorder. This consists, in principle again, of a number of valve stages in which for every two pulses received one is transmitted on to the next. If you have say 5 of these stages then for every 32 pulses received by the counter which are amplified and passed on to the scaler, only one will be passed out of the scaler and operate the mechanical recorder. Very fast rates of counting can thus be accommodated by such an instrument. The actual number of pulses of course is obtained by multiplying the scale reading by the scaling down factor.

In practice the counter tube is usually supported vertically with the thin window downwards, in the middle of a lead block, appropriately called a lead castle, and the specimen to be examined for its radioactivity spread out in a small dish located directly under the end window. The lead castle fulfils at least two functions. Its thick lead walls exclude high voltage interference from neighbouring equipment, and it can also be utilised to fix the relative position of the counter and dish holding the specimen. Lead castles commercially supplied are provided with shelves, so that by working always on the same shelf, it is possible to standardise the conditions and to ensure that in every experiment the same proportion of the total number of $\beta$ particles given off is counted. All this applies to counting $\beta$ particles emitted from a solid sample. Special

counters have been designed to measure the radioactivity of radioactive material in solution. The counting efficiency of such tubes is less than that of the dry counters, because some of the $\beta$ particles are absorbed as they pass through the solution.

There is one other way in which radioactivity is made to manifest itself, and that is by allowing the radiations to affect a photographic film, utilising Becquerel's observations. This is the basis of the technique of radioautography which is rapidly becoming of very great importance. It has not yet been placed on a quantitative basis, but it is proving of great value in locating radioactivity in some parts of a leaf, or indeed of a whole plant. In principle the procedure is to treat a plant with a radioactive solution, say radioactive phosphate, and then after a certain time to place the photographic plate in very close proximity to that part of the plant selected for examination and allow the areas in which the radioactivity is concentrated to fog the plate, which is then developed. Most of the refinements with this technique, however, have been carried out on vegetable or animal tissue which has been sectioned. It is a technique which is likely to yield a wealth of information both to the plant nutritionist and the physiologist.

**Application of Tracer Technique to Plant Nutrition Problems.**

We are now in a position to consider what tracer technique really is. For simplicity let us consider a particular isotope $P^{32}$, and to fix our ideas I shall use as an illustration the experiment we carried out at Long Ashton last summer principally to acquire tracer experience. When a plant grows in soil which has been treated with fertiliser, the plant absorbs nutrient, both from the soil and fertiliser, but once the nutrient has entered the plant it is normally impossible to say whether it was taken from the soil or from the fertiliser. It was to obtain information on this relative contribution of soil and fertiliser to rapidly maturing plants on various soils that the experiment was designed.

Since we wished to use the radioactive isotope of phosphorus, $P^{32}$, phosphate was the particular nutrient which was followed.

The procedure was to send some A. R. disodium hydrogen phosphate $Na_2HP^{31}0_4$ to Harwell. This was put in the Atomic Pile, and as a result of the neutron bombardment, the powder removed from the Pile two or three weeks later was still mostly $Na_2HP^{31}0_4$, but some of the $P^{31}$ atoms in the phosphate molecules had been hit by neutrons and so converted into $P^{32}$ atoms. This meant that there was about one molecule of $Na_2HP^{32}0_4$ per 1000 million or more of $Na_2HP^{31}0_4$ molecules. These two types of molecules were identical in chemical properties and there is no reason to believe that the plant could discriminate between them. As stated previously the sole difference between them is that the nucleus of each $P^{32}$ atom has one more neutron in it than its stable analogue $P^{31}$. These $Na_2HP^{32}0_4$ molecules are unstable, however, some of the $P^{32}$ atoms in them decomposing all the time with the ejection of an electron—in such numbers that at the end of a ' half life,'—which is 14 days for $P^{32}$ –the number of $Na_2HP^{32}0_4$ molecules will be exactly half the number there were at the beginning of this period.

When we received the so-called radioactive sodium phosphate from Harwell, it was dissolved in water, and this was the solution applied to the plants as fertiliser phosphate. The other nutrients were applied normally, but the whole phosphate was added at the time of sowing the seeds—a known volume added to each pot in which the seeds were sown.

Suppose the composition of the radioactive phosphate solution to be :—

$Na_2HP^{31}0_4$—say 50 g./litre or 50 mg./ml.
$Na_2HP^{32}0_4$—negligible wt.

Suppose one ml. of this solution on evaporation to dryness in a standard counting dish gives say 1000 counts/min. In this solution therefore 50 mg. of $Na_2HP^{31}0_4$ are intimately associated with 1000 counts/min. radioactivity. If we added 10 ml. of this solution to each pot, we should be adding 500 mg. of $Na_2HP^{31}0_4$ intimately mixed with $Na_2HP^{32}0_4$ which although present in infinitesimal amounts would still reveal its presence by giving out 10,000 counts per min. In other words, if we neglect the decay of the $P^{32}$, this 500 mg. $Na_2HP^{31}0_4$ given to each pot will always be associated with a known radioactivity—10,000 counts per min. If the plant takes in some of this, and some phosphate from the soil as well, the radioactive tracer or fertiliser phosphate in the plant can easily be determined by ashing the plant and determining the number of counts in the ash. If we recorded 10,000 counts per min. from this ash we would know that all the $Na_2HP^{31}0_4$ associated with such a rate of counting, i.e. 500 mg., had been absorbed in the plant. If the number of counts observed were 100 per min., then we would know that only 1% of the tracer phosphate, that is the fertiliser phosphate, had entered the plant. A simple additional test to determine the total phosphate in the plant would at once enable us, by difference, to say how much phosphate had come from the soil. In such experiments as this the decay of the radioactive isotope can be allowed for quite easily.

A large number of plants were grown and some were cut at 3, 6, and 9 weeks after sowing, so that we should have found the relative phosphate intake from soil and fertiliser at different stages in the plant growth. However, only if the applied fertiliser contained the two phosphorus isotopes $P^{31}$ and $P^{32}$ in the same chemical form—in this case as orthophosphate—could it be assumed that the plant would not be discriminative. Unfortunately, we found that some of the radioactive sodium phosphate molecules had been decomposed to sodium phosphite during irradiation in the pile, so that we had applied the $P^{31}$ and $P^{32}$ in different chemical forms to the plants, which did not conform to the above requirement regarding similarity of chemical form. Consequently, since it could not be assumed that the plants would treat indiscriminately the orthophosphate and phosphite molecules, our data were incapable of interpretation.

This is only one very simple example of the use of $P^{32}$ as a tracer. The possibilities are extremely numerous, both with phosphorus and with other elements equally important in plant growth. In most cases, a tracer experiment must be preceeded by a laboratory synthesis of the compound it is desired to follow, incorporating the radioactive isotope in order to 'label' or 'tag' the molecule.

**Factors Limiting Usefulness of Isotopes for Biochemical Research.**

A few general points will be listed regarding the factors controlling the usefulness of available isotopes. Should we wish to follow the entry of a particular element into the plant system, we must know whether a suitable radioactive isotope of that element is available. The first point to look for is the half life period quoted (in the published tables); in general an experiment can be made to last about 6 or 8 half lives. In other words an experiment with $P^{32}$ could last about 3 to 4 months, whereas an experiment

with sodium (half life 15 hours) could last only a few days. After that time, because of the decay of the radioactivity, the measuring of the residual activity would become too tedious. If on the other hand the half life is very long, then because of, shall we say, the persistency of the element, much more rigorous handling precautions are necessary. If one spills some on the floors, the radiation from it will still be given out long after the culprit has ceased to have any further terrestial interest in it!

The other desirable consideration besides suitable half life is that the isotope in question should be a powerful $\beta$ emitter; in other words, the particles which are emitted should be energetic ones. If this is not so, many of them would find difficulty in penetrating the very thin metal film window of the counter, and special procedures would be required to count them.

With reference to the effects of the radiations emitted by radioactive isotopes both on the plants and on the person carrying out the tests, the best approach is to regard all radiation as harmful—excessive radiation dosages in plants may cause chromosome breaks leading to a definite mutation. This has been found of use in deliberately producing new strains. In our work here, however, we wish to minimise this radiation effect, striking a balance between this and the requirement to apply sufficient radioactive isotope to last the duration of the experiment.

Regarding health hazards to human beings, in general $\beta$ emitters are not normally very dangerous in the order of activities used in tracer work—unless they are consumed through the mouth or inhaled into the lungs, when they may subject local internal parts of the body to prolonged radiation. This is especially so if they tend to have fairly long half lives. Scrupulous cleanliness and the avoidance of dust by having good ventilation is essential.

## SUMMARY.

1. A simplified description of atom structure is given, leading to the term ' radioactive isotope.' Some of the properties of the principal radiations emitted by radioactive elements are discussed.

2. The principles underlying the qualitative and quantitative measurement of radioactivity are indicated, and the Geiger Muller Counter Method outlined.

3. The application of radioactive tracer technique to plant nutritional studies is illustrated by means of a simple example.

# THE SUPPRESSION OF SPROUTING IN WARE POTATOES.

By L. C. LUCKWILL.

## INTRODUCTION.

Experiments on the use of growth-substances to suppress the sprouting of ware potatoes in storage were continued and extended during the season 1947-48. In addition to methyl-alpha-naphthyl acetate (MANA) and methyl-alpha-naphthyl methyl ether (MNME), which the previous season's trials had shown to be highly effective in suppressing sprouting (3, 4), a third substance, 2-3-5-6-tetra-chloro-nitrobenzene (TCNB), was tested. This substance, which is used as a fungicide for the control of dry rot in seed potatoes, has been shown by Brown (1) to be active also as a sprout-depressant. Although it cannot be considered as a growth-substance within the usual meaning of the term, it was considered of interest to compare its sprout-inhibiting properties with those of MANA and MNME when used at similar rates of application. Like the other two substances, TCNB has a fumigant action and can be applied on a dust or paper "carrier" from which it vapourises slowly during the period of storage.

A further object of the 1947-48 trials was to investigate the responses of different varieties of potatoes to these treatments, and for this purpose Kerr's Pink and Arran Banner were chosen as representative of early-sprouting maincrop varieties, and Majestic as a late-sprouting variety.

## EXPERIMENTAL METHODS.

*Storage.* As in the previous season's work storage was again in half-bushel wooden fruit boxes, loosely lined with greaseproof paper. Six kilograms of potatoes were weighed into each box and, after treatment, the boxes were closed and then stacked on a layer of straw placed over the concrete floor of a cool outbuilding. As a protection against frost the stack was covered with straw and was surrounded on all sides with a wall of straw bales. As the boxes were not air-tight it was considered advisable to stack the control boxes and the various treatments separately, thus obviating the possibility of leakage of vapour into the untreated boxes.

*Treatment.* Prior to storage the potatoes were hand-graded. Only medium sized tubers were used in the experiment and, after washing and drying, these were carefully selected for freedom from disease.

In this experiment paper confetti was used as the "carrier" for the sprout depressants. For ware potatoes this has the great advantage that it does not adhere to the surface of the tubers in the same way that dusts such as talc or kaolin do, and is much easier to distribute than the paper strips used in a previous experiment.

The confetti was impregnated with 1% of its weight of sprout-depressant. This was done by dissolving the requisite amount of the chemical in acetone and spraying it on to a thin layer of the confetti, which was frequently turned during the spraying, and was afterwards thoroughly mixed. The potatoes were treated by scattering the required amount of impregnated confetti amongst them after they were in the boxes.

Each of the three chemicals used was tested at three different rates of

application, corresponding to doses of 20, 50 and 100 gm. of the chemical per ton of potatoes. In addition there was an untreated control set in each series. Each treatment was represented by three replicate boxes, so that the general arrangement of the experiment was as follows :—

3 varieties × 3 substances × 4 rates × 3 replicates = 108 boxes.

The potatoes were placed in the boxes and treated on December 3rd, 1947. From March 1948 onwards control boxes of each of the three varieties were inspected from time to time in order to assess the progress of sprouting. As sprouting was most advanced in Kerr's Pink, this variety was sampled first, on April 22nd. The boxes containing Arran Banner were opened on April 30th, the later sprouting Majestic being kept in store until May 28th.

As each box was opened the potatoes were desprouted and the total fresh weight of sprouts produced in each box was determined. The tubers themselves were then weighed to the nearest 5 gm. (0·09%) in order to estimate the loss in weight due to evaporation and respiration during the period of storage.

## RESULTS.

*Sprout weight.* The mean fresh weight of sprouts produced per box of six kilograms of potatoes was taken as a measure of the degree of sprouting and the results for the three different varieties are summarised in Table I.

It is clear from this table that the varieties used did not all respond in the same way to the three different sprout-depressants. In particular, it will be noticed that TCNB, which was very effective in suppressing the sprouting of Arran Banner, was relatively ineffective on Kerr's Pink ; and that MANA, which was the most active substance on Majestic, was the least active of the three on Arran Banner. This point is brought out even more clearly by Fig. 1 in which a direct comparison of the three varieties has been made possible by expressing the weight of sprouts produced as a percentage of the mean weight produced by the untreated controls.

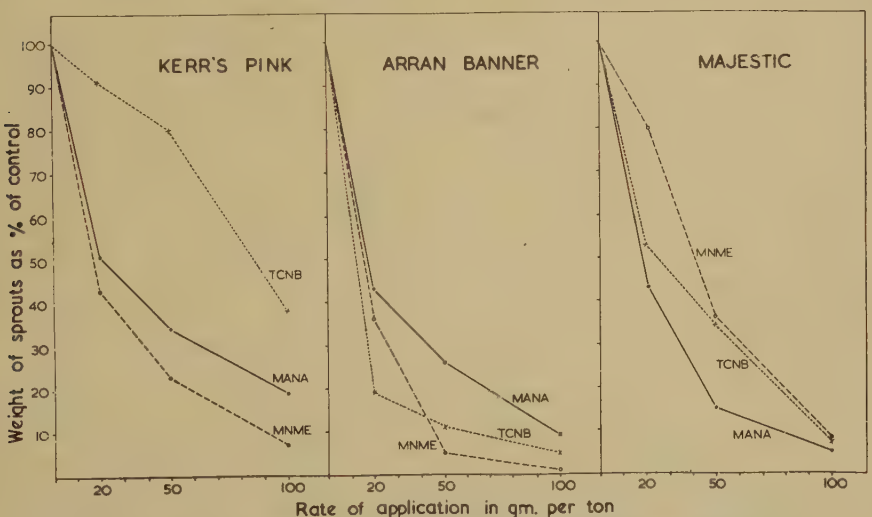


*Fig.* 1. The effect of three different substances (TCNB, MANA and MNME) on sprout production of stored potatoes.

The sprout weight data were analysed using the technique of Analysis of Variance. For this analysis the logarithms of the sprout weights were

used, as the distribution of these approximates more closely to normal than does that of the sprout weights themselves. The statistical significance of the differential responses noted above is confirmed by the significant interaction variances, particularly that of Variety × Substance (Table II).

TABLE I.

Mean Fresh Weight of Sprouts in gm. per 6 kilos of Potatoes. (Each Figure in the Table is the Mean of Three Samples).

| Variety | Substance | Rate of application (gm./ton) | | | |
|---|---|---|---|---|---|
| | | 0 | 20 | 50 | 100 |
| Kerr's Pink .. | MANA | 285 | 157 | 104 | 59 |
| | MNME | 251 | 132 | 71 | 20 |
| | TCNB | 379 | 276 | 245 | 117 |
| Arran Banner .. | MANA | 438 | 148 | 89 | 32 |
| | MNME | 258 | 122 | 17 | 5 |
| | TCNB | 327 | 66 | 39 | 16 |
| Majestic .. | MANA | 264 | 114 | 39 | 14 |
| | MNME | 278 | 212 | 95 | 21 |
| | TCNB | 248 | 139 | 89 | 18 |

TABLE II.

Analysis of Variance of Log. Sprout Weights.

| | Sum of squares | df | Mean square | F | 1% F |
|---|---|---|---|---|---|
| Variety .. .. .. .. | 2·1059 | 2 | 1·0529 | 33·4 | 4·92 |
| Rate .. .. .. .. | 19·4649 | 3 | 6·4883 | 206·0 | 4·07 |
| Substance .. .. .. | 0·6476 | 2 | 0·3238 | 10·3 | 4·92 |
| Variety × rate .. .. .. | 1·3319 | 6 | 0·2219 | 7·0 | 3·07 |
| Variety × substance .. .. | 2·8401 | 4 | 0·7100 | 22·5 | 3·60 |
| Substance × rate .. .. | 0·4390 | 6 | 0·0731 | 2·3 | 3·07 |
| Variety × substance × rate .. | 0·8264 | 12 | 0·0689 | 2·2 | 2·45 |
| Remainder (Error) .. .. | 2·2703 | 72 | 0·0315 | — | — |
| Total .. .. .. .. | 29·9261 | 107 | — | — | — |

In general, it may be said that Arran Banner responded more readily to all the sprout depressants used than did Kerr's Pink, although both may be classed as early sprouting varieties. The late sprouting Majestic was intermediate in its response.

As in previous experiments the appearance and general quality of the samples in which sprouting had been inhibited was better than that of the untreated controls. The treated potatoes were still firm and hard to the

touch, whereas those in which excessive sprouting had occurred were flabby in texture and appeared considerably shrivelled.

*Loss in weight.* The loss in weight of potatoes during storage, usually referred to as " shrinkage," may be due to a number of causes which can be broadly classified as " physiological " and " pathological." Owing to careful selection of the material used, pathological shrinkage, due to storage rots, was entirely absent from this experiment : the losses in weight recorded may therefore be considered as due entirely to physiological causes. Here, the most important factor is loss of water from the tuber. Once the skin of the potato has hardened (i.e. after the initial " sweating " period is over) water is lost chiefly through the sprouts, and might therefore be expected to be largely a function of sprout weight. The total decrease in weight of the potatoes, prior to the removal of the sprouts includes also the losses of food material due to respiratory activity during the period of storage. The combined losses, due to these two causes, for the samples treated at the highest rate of application of each of the three chemicals, are shown in Table III.

TABLE III.

Percentage Loss in Weight of Potatoes Treated With Three Different Sprout-Depressants at the Rate of 100 gm. Per Ton.

| Variety | Substance | % loss due to : | | Total " shrinkage " % |
|---|---|---|---|---|
| | | Evaporation & respiration | Removal of sprouts | |
| Kerr's Pink .. | MANA | 10·0 | 1·0 | 11·0 |
| | MNME | 7·3 | 0·4 | 7·7 |
| | TCNB | 8·8 | 2·0 | 10·8 |
| | Control | 8·4 | 5·1 | 13·5 |
| Arran Banner .. | MANA | 5·4 | 0·5 | 5·9 |
| | MNME | 4·3 | 0·1 | 4·4 |
| | TCNB | 5·0 | 0·2 | 5·2 |
| | Control | 6·8 | 5·7 | 12·5 |
| Majestic .. | MANA | 5·3 | 0·2 | 5·5 |
| | MNME | 5·2 | 0·3 | 5·5 |
| | TCNB | 6·2 | 0·3 | 6·5 |
| | Control | 8·0 | 4·4 | 12·4 |

It will be noticed from the above table that the loss in weight of the treated samples was much greater than might have been expected in view of the considerable inhibition of sprout development achieved. Thus, in the Majestic samples treated with MANA at the rate of 100 gm. per ton the mean sprout weight was only 5% of that of the untreated control samples ; water and respiratory losses, on the other hand, were 66% of that of the controls. In Kerr's Pink the potatoes treated with MANA actually lost more weight during storage than those not treated, although they produced only 19% of the weight of sprouts. Although this increase is barely significant at the 5% level the data suggest strongly that treatment with sprout-depressants, particularly MANA, may actually stimulate respiratory activity in the stored tubers, and that the losses due to this increased respiration may, in some cases, cancel out the gains resulting from sprout inhibition and reduced water loss.

From the practical point of view it is the total " shrinkage " which is chiefly of importance. This is the loss in weight of the potatoes after de-sprouting and includes not only respiratory and water losses, but also losses due to removal of materials used in the growth of the sprouts. In

all cases the total shrinkage of the treated samples was less than that of the controls, though for Kerr's Pink treated with MANA and TCNB the difference between the treated and untreated samples was not statistically significant.

It is of interest to note that even where the total loss in weight of the treated tubers approached that of the controls (e.g. Kerr's Pink treated with MANA at 100 gm./ton), the greater firmness of the treated samples was still very marked. This suggests that the shrinkage of the untreated tubers was mainly due to water loss through the sprouts, whereas that of the treated tubers resulted chiefly from loss of food material through high respiratory activity.

Direct evidence that the respiration of stored potatoes may be increased by treatment with growth substances has been obtained by Ulrich (5), who measured the carbon dioxide output of potatoes treated with Rhizopon C, a proprietary form of MANA. Although the rate of application is not stated, it is clear from the published graph that the respiration rate of the treated samples was 30—50% higher than that of the untreated controls over the period of the experiment, which lasted from April 10th—June 25th. Similar experiments are now in progress at Long Ashton.

## DISCUSSION.

In this experiment all three chemicals tested were active in suppressing the sprouting of potatoes stored in wooden boxes. No particular substance can be claimed as the most effective, since their relative activity varied both with the variety of potato and the rate of application.

As has been emphasised in previous reports, however, the possibility of applying these treatments on a commercial scale is governed largely by economic considerations. What is of importance from the practical viewpoint therefore, is not primarily the relative effectiveness of the different chemicals, but the cost of the treatments relative to the price of ware potatoes.

An attempt to assess the relative costs of the three different treatments is presented in Table IV. A reduction in sprout weight to the level of 20% of that of the untreated controls has been chosen as a desirable " target " at which to aim, and the rates of application necessary to achieve this result, under the conditions of the experiment, have been estimated from the curves in Fig. 1.

TABLE IV.

Estimates of Relative Costs of Sprout-Inhibiting Treatments for Indoor Storage of Potatoes.

| Variety | Substance | Rate of application necessary for 80% reduction of sprouting. (gm./ton) | Approximate cost of material (commercial grade bulk purchase). (Shillings/100 gm.) | Cost of material required to treat 1 ton of potatoes. (Shillings) |
|---|---|---|---|---|
| Kerr's Pink .. | MANA | 96 | 30 | 28·8 |
| | MNME | 56 | 15 | 8·4 |
| | TCNB | 210* | 1·2 | 2·5 |
| Arran Banner .. | MANA | 62 | 30 | 18·6 |
| | MNME | 30 | 15 | 4·5 |
| | TCNB | 19 | 1·2 | 0·23 |
| Majestic .. | MANA | 40 | 30 | 12·0 |
| | MNME | 76 | 15 | 11·4 |
| | TCNB | 68 | 1·2 | 0·82 |

* Estimated by extrapolation of the sprout-weight/dose curve.

It must be clearly understood that the prices quoted for the three chemicals are the current ones for commercial grade material purchased in bulk, and that the cost to the grower of these sprout-depressants, ready mixed with a suitable carrier and marketed through the usual trade channels, would be considerably greater than shown in the table.

It is quite clear however that TCNB, on account of its cheapness, has a great advantage over the relatively expensive naphthalene compounds, and that it is the only one of the three treatments which at present fall within the range of economic possibility. This is true even for varieties such as Kerr's Pink, which require a high rate of application in order to suppress sprouting.

TCNB treated potatoes have been fed to pigs, poultry and rabbits without apparent ill effect and it seems that this compound is probably of a very low order of toxicity. Confirmatory evidence of this, however, would obviously be desirable before applying the treatment on a large scale to potatoes intended for human consumption.

Further information is also required on the performance of these sprout inhibiting substances in potato clamps, where the conditions are often quite different from those obtaining in indoor storage. Recent small scale experiments by Glasscock (2) have shown that certain sprout depressants such as MNME, which are highly effective in indoor storage, may, under certain conditions, actually stimulate sprouting in the clamp. The reason for discrepancies of this type is not at present clear: the more rapid dispersion of volatile substances as a result of greater ventilation in the clamp as compared with indoor storage is undoubtedly an important factor, and the possibility of the inactivation of the sprout-depressant by soil micro-organisms must also be borne in mind.

## SUMMARY.

(1) The experiment was designed to compare the effectiveness of three different chemicals (MANA, MNME and TCNB) in suppressing the sprouting of ware potatoes stored in wooden boxes.

(2) All the substances used proved active in suppressing sprouting, but their relative activity varied with the variety of potato used and with the rate of application.

(3) TCNB has the great advantage that it is much cheaper than either MANA or MNME.

(4) The loss in weight of some of the treated samples was as great as, or even greater than, that of the untreated controls, even although sprouting had been suppressed. It is suggested that this may be due to a stimulation of respiration as a result of treatment.

(5) The need for further information on the performance of sprout-depressants under clamp conditions is stressed.

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# AN UNDERGROUND RETURN CIRCUIT SPRAY MAINS SYSTEM SUITABLE FOR 15-25 ACRES OF TOP FRUIT.

By H. G. H. KEARNS and N. G. MORGAN.

It is recognised in all countries producing first quality fruit, particularly apples, that the control of pests and diseases is the most important and most expensive feature of the year's work. A failure to ensure a very high degree of control completely renders all other operations of little value for the economic production of a crop.

Spray programmes have been worked out for the control of nearly all the most important insect pests and diseases of fruit under varying climatic conditions in Great Britain. The large quantities of poor grade apples produced is almost entirely due to the unsuccessful application of proven control measures. The position could be rectified in most cases if spraying equipment of sufficient capacity to spray the crop at the essential stages of growth was installed. In the case of apples and pears it is essential that the spray equipment and personnel are of such capacity that under the worst climatic conditions the entire crop can be sprayed every ten days from bud burst until three weeks after petal-fall. At petal-fall of apples the thoroughness of the application must be of an order that ensures complete wetting of the inside of every calyx cup and the underside of every leaf. At all other stages the coverage of the buds, leaves and fruitlets must be of a very high order but not so complete as at petal-fall.

The formulations of spray fluids have been based on the use of considerable amounts of water as a vehicle. This procedure automatically provides the grower with a safety factor of " run off " and the large volume materially assists in obtaining an adequate coverage of the target. The hard labour and time involved in the use of these large volumes have promoted investigation of less laborious and more rapid methods of application such as " automatic " spraying. Although developments aiming at the application of small volumes of highly concentrated materials are in progress it seems that the use of large volumes of spray fluids on top fruit is likely to remain the standard method for a considerable period for a number of technical reasons. There are a number of methods of applying large volumes of spray fluids to top fruit, each method offering advantages. These include hand spraying with " brooms " from spray mains or mobile outfits and various forms of " automatic " spraying machines. The latter very considerably reduce the hard labour associated with hand spraying but they are often ill-suited for the efficient spraying of many existing orchards of wide and tall bush trees. Unfortunately the efficient " automatic " machines with their ancillary equipment are large and expensive and therefore are not suitable for the small and medium sized plantations which form the backbone of English fruit growing. The small " automatic " spraying machine is of very limited use on present day type of trees.

The ever increasing cost of labour on fruit farms renders it essential to obtain the greatest returns for the minimum of effort. The spray mains system, either overhead or underground, offers a method of spraying efficiently in small orchards, especially those on hill slopes laid down to grass. In this paper a system of underground mains is described which fits in with some

orchards better than alternative methods. It is emphasised that each orchard presents its own problem of spraying and the equipment must be matched to suit the local circumstances. No system of spraying is greatly superior to all others.

**Broad specification of spray mains systems.**

Underground spray mains systems have been installed for many years in large orchards. Until the advent of large automatic sprayers they offered without question the best method of rapid spraying and they still offer the best engineering lay-out. Some systems have given trouble due to faulty lay-out of the piping circuits, which has accelerated the rate of extensive spray material deposit within the pipes and subsequent increase in frictional loss of pressure. Others have been subject to extensive and rapid exterior corrosion of the piping due to local soil conditions. Most systems do not provide sufficient stand pipes (take-off valves) per acre to permit the use of reasonable lengths of rubber spray hose. The problem of blocking up by deposit within the pipes can be largely overcome by an arrangement that the spray fluid is constantly on the move at a reasonably high velocity and the fluid not used for supplying the spray lances is returned to the pump house. The return circuit in this case is fitted with a relief valve which only operates at a pressure in excess of that required on flow at the lance.

The problem of external corrosion of pipe in some soils, particularly clays, can be partially overcome by choice of suitable pipe protectants but the better solution is to erect the pipe system overhead. This method has other advantages in that leaking joints etc. can be readily detected and repaired.

A bush orchard in full bearing with trees of up to 15 feet in height will require about 500 gallons of wash per acre per application. A sprayman in a working day of six hours will apply from 1,000—1,800 gallons of wash, the volume depending on the soil conditions, size of disc and labour involved in hose pulling. It may be assumed that two operators using spray brooms should spray about 4 acres per day. So the maximum number of acres that can be sprayed by the two men in the prescribed spray period of ten days is 40 acres, provided the weather permits of continuous spraying. This procedure is impracticable as the weather is not likely to be suitable for spraying during the whole period.

The choice of a pump capacity for 25 acres is based on the maximum economic output of two lances (1). The output of two lances is 10-16 g.p.m. Therefore the pump capacity must be adequate to supply this volume at a minimum flow pressure at the lance of 250 lb. per sq. in. It is desirable in selecting the pump capacity to allow a margin for wear of valve assemblies and other factors. It is recommended that with a maximum total lance output of 16 g.p.m. a pump of not less than 20 g.p.m. is used.

In the following account a description is given of an underground mains system, incorporating a number of improvements, which was designed for and installed in a 15 acre top fruit plantation.

**The Pump House and Water Reservoir.**

A small pump house was erected 10 feet wide, 16 feet long and 9 feet high to the eaves. Adjoining the house a sunken cement water reservoir was made to hold approximately 2,500 gallons. The pump house was made of breeze blocks, rough cast outside and roofed with tiles. Window casements were provided in the walls to ensure adequate light. Within the house three

sunken cement tanks were made, each of a capacity of 300 gallons: two of the tanks were intended for the diluted wash and the third for the agitation of oil emulsion concentrates, or alternatively as a third dilution tank. The cement of the tanks was carefully compounded to avoid porosity. The tanks were provided with overflow gulleys and the cement floor levels arranged for easy washing down and drainage. The pump house was provided with a cement path outside and an unloading ramp for barrels.

**The Pump House Equipment.**

Within the pump house the pumps and ancillary equipment were installed in a compact yet accessible manner. Particular care was taken so that every item could be readily taken apart for repair. The pumps were driven by 3-phase electric motors. Automatic control gear for the pumps was not used as the size of the installation did not warrant the expense or complication. Electric lighting and inspection lamps were provided. The principal items of equipment are as follows:—

(1) Three-ram totally-enclosed medium-speed pressure pump rated at 20 g.p.m. output at up to 600 lb. per sq. in. pressure. The pump is driven by a 15 h.p. electric motor.

(2) A self-priming centrifugal pump with a capacity of 40-50 g.p.m. delivery, direct driven by a $1\frac{1}{2}$ h.p. electric motor. This pump lifts water from the reservoir to the dilution tanks, agitates the wash within the dilution tank and when necessary feeds the suction line of the high pressure pump.

(3) Electric manually operated control gear for the two pumps.

(4) A 12 g.p.m. semi-rotary pump for supplying concentrates to a measuring tank.

(5) The pipe circuits (described below).

(6) A workshop bench with suitable tools for routine repairs.

**The Pump House Pipe Circuits.**

The lay-out of the pipe circuits within the pump house is shown in the schematic drawing (*Fig.* 1). It may be conveniently divided as follows:—

(a) Water supply to reservoir.

(b) Suction circuit from reservoir to fill spray dilution tanks with water.

(c) Agitation circuit to spray dilution tanks and by-pass to suction circuit of the high pressure pump.

(d) Suction circuit of the high pressure pump.

(e) Delivery circuit of the high pressure pump.

(f) Overflow circuit from the relief valve assembly on the high pressure pump.

(g) Overflow from the relief valve of the return circuit.

(h) Spray concentrate circuit.

(a) *Water supply to reservoir.* It was assumed that between 2,000—3,500 gallons of water would be required for a day's spraying. Spring water was available and the inflow during the night and day was normally sufficient to supply this volume. A one-inch galvanised supply pipe was laid 15″—18″ below soil level from a small hillside reservoir and the water flowed by gravity to the pump house. The pipe entered the pump house through the floor and made an exit through the wall to a large 1″ B.S.P. ball valve fitted in an accessible position on a side wall of the reservoir. A stop cock was fitted to permit easy repair of the ball float valve and a hose tap was fitted between

the cock and the valve to permit of easy draining and thereby avoid all risk of frost damage. An emergency stop cock was also fitted at the source of supply of the water.

(b) *Suction circuit from the water reservoir.* The water is conveyed from

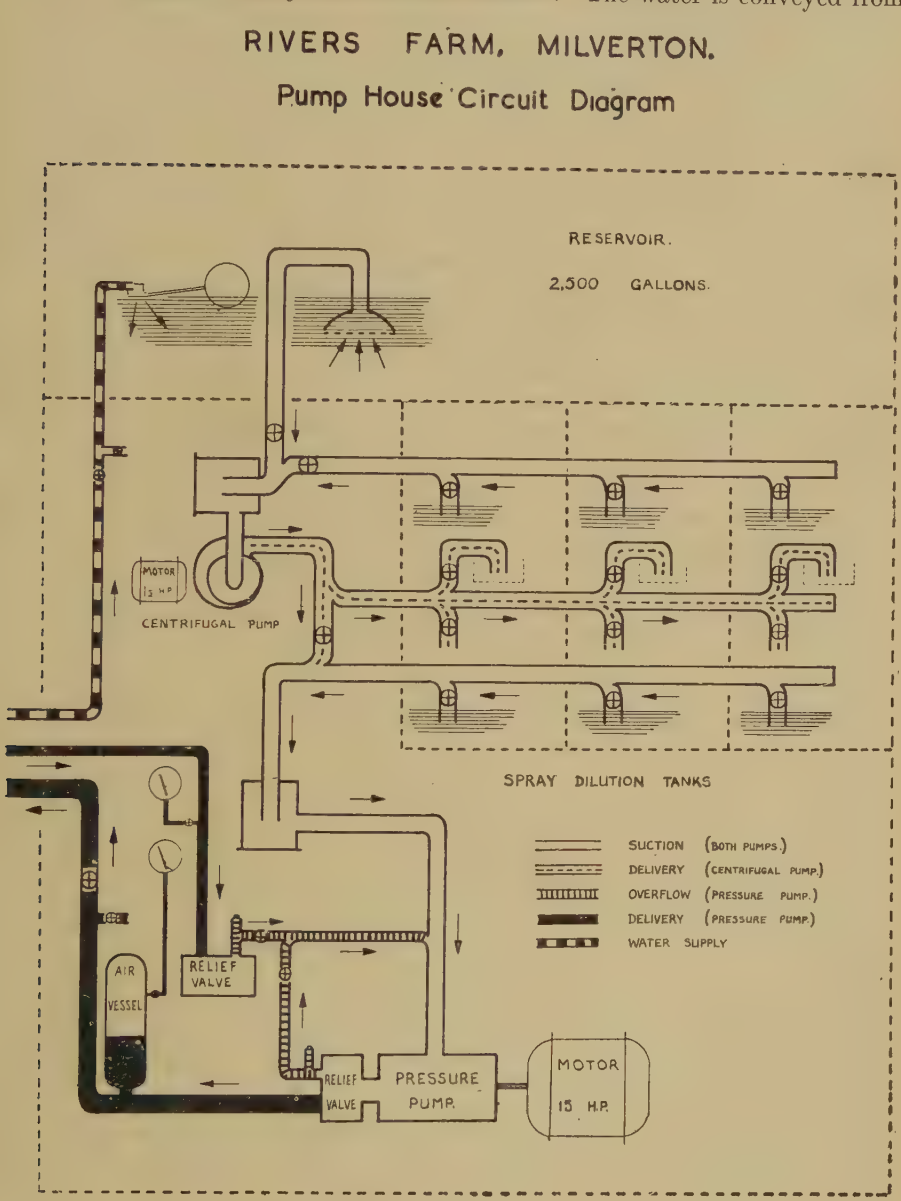


*Fig.* 1.

the reservoir to each of the spray dilution cement tanks by means of a centrifugal pump. The $1\frac{1}{2}''$ B.S.P. suction pipe is fitted with a $10'' \times 3''$ heavy gauge galvanised mild steel suction filter with entry holes of $\frac{3}{16}''$ diameter. The suction pipe can be readily removed from the reservoir for cleaning the filter. The water before entering the centrifugal pump passes through an $8'' \times 4''$—16

mesh phosphor-bronze filter cylinder inside a filter pot. The water is pumped to one of the cement tanks via an overhead filter tank of 25 gallons capacity. Each filter tank is fitted with four 6-inch diameter circular filter gauzes with a mesh of 24 apertures per linear inch. The gauze is soldered over an area closely drilled with 1″ circular holes. The inflowing water discharges on an area of the filter tank so that the gauzes are protected from damage by the pounding force of the large volume of water. The filter tanks are readily removed for cleaning with a spray gun. The suction and delivery of this circuit are through 1½″ B.S. pipe and are controlled by fullway wheel-type gun metal valves.

(c) *Agitator circuit and by-pass to high pressure pump.* The diluted wash from a cement tank is pumped via the centrifugal pump and returned to the bottom of the tank. This procedure avoids or reduces appreciably the formation of " foam " which would be caused by discharging through an air space on top of the fluid, as the formation of foam causes air locks in the high pressure circuit.

A by-pass from the delivery circuit can be used to feed the suction circuit of the high pressure pump, a feature that is particularly useful when a bucket type plunger pump is started up. Generally a quick change-over can be made to the normal unassisted high pressure pump suction circuit.

Generally it is unnecessary to run the agitation circuit continuously. It is only when materials do not remain too well in suspension that continuous agitation is necessary. The circuit is controlled by suitably placed 1½″ B.S.P. fullway wheel valves.

(d) *Suction circuit of the high pressure pump.* The high pressure pump is called upon to lift the fluid a maximum of 6 feet from the floor tanks. A pump fitted with single cup type bucket plunger and which has not been in use for some days may fail to maintain a vacuum on the suction stroke ; it is therefore necessary to prime it by fitting a tank above it or by the method described in the previous section. Generally after the pump has been running for about ten minutes the buckets have become pliable and lubricated and a change over from the force feed to normal suction is possible and satisfactory. The force feed from the centrifugal pump may be necessary when the buckets are beginning to fail due to wear which causes a drop in pressure. The use of force feed generally rectifies the loss of pressure within a few minutes.

(e), (f) and (g). *Delivery circuit of the high pressure pump and overflow circuits.* In a mains system loss of pressure occurs due to pipe friction and the normal flow losses caused by discharge. It is highly desirable, however, that the wash delivered at the nozzle shall maintain constant pressure as this assists materially in obtaining uniformity of spray pattern. The pump must therefore be fitted with an efficient relief valve that maintains this even pressure. This relief valve also protects the pump and pipe circuit. In a return circuit system the volume of wash unused by the nozzles is returned to the pump house and is discharged into the tanks or suction circuit via a second adjustable relief valve. This arrangement prevents the build-up of excessive pressures due to an accidental closing of the delivery mains or return circuit by a valve or blockage.

The two relief valves are adjusted to release on the return valve so that the flow pressure at the lances is not less than 250 lb. per sq. in. It should be noted that an efficient relief valve assembly should permit the pump at this flow pressure to deliver a volume not appreciably lower than its volumetric displacement at 50 lb. per sq. in. pressure.

The assembly of the high pressure circuit in the pump house should be arranged, as shown in the schematic drawing of the lay-out, so that the volumetric displacement of the pump and the overflows from the two relief valves can be readily and separately ascertained. The pressure gauges must be accurately calibrated and protected from vibration and sudden pressure shocks. This is best and conveniently arranged by mounting the two gauges side by side on a panel away from vibrating pipework, the gauge in each case protected by a hand-operated needle valve and a high pressure oil-resistant rubber hose connection. One gauge is connected to the air vessel fitted in the delivery pipe near the pump and the second to the return circuit pipe before it joins the relief valve. The pressure gauges should not be in constant use and should only be used as testing instruments when adjusting or checking pressures or tracing a fault.

(h) *The spray concentrate circuit.* With some types of concentrates, more particularly oil emulsions that have been stored for considerable periods, it is desirable to mix them thoroughly before dilution. This procedure can only be carried out by emptying the barrel and agitating the contents with a perforated plate type plunger or by passing through a gear-type pump. It is also a convenience when handling large volumes of concentrate to pump the liquid to a measuring device.

In the pump house described, one of the 300-gallon cement tanks is used for this purpose. The full barrels of wash are rolled along a raised cement ramp outside the house and the contents discharged into a large glazed gulley which leads into the tank.

The concentrate after agitation with a plate plunger is pumped by a large semi-rotary pump to a measuring tank which discharges into the dilution tanks. Provision is made for the easy washing of the gulley, the concentrate tank and the semi-rotary pump by simple hose connections to the centrifugal pump. In many cases it is more convenient to measure out the concentrates by hand.

**Lay-out of the Pipe Circuit in the Orchard.**

The lay-out of the pipe circuits in a 15 acre orchard is shown on the plan. (*Fig.* 2). The principal features are as follows :—

(1) The gradient of the land slopes downwards from North to South, the pump house is sited so that the maximum height of the spray main is 30 feet above it and the fall 60 feet.

(2) The principal spray main is 864 feet of $1\frac{1}{2}''$ bore pipe, excluding 50 feet approx. service pipe from the main pump-house, and it is in a straight line.

(3) Laterals of $\frac{3}{4}''$ bore pipe are arranged at right angles to the mains at intervals of 108 feet. The laterals are on each side of the main and in line with each other.

(4) Each lateral is controlled at each end by a $\frac{3}{4}''$ fullway valve with a key head.

(5) The ends of the laterals join a $\frac{3}{4}''$ bore pipe which forms the return circuit to the pump house.

(6) For the sake of convenience in operating the lay-out in the particular orchard, the complete circuit is divided into four sub-circuits each of which can be operated separately.

(7) Stand pipes, each with a control valve, are sited 108 feet apart along each lateral.

(8) The spacing distance of 108 feet between laterals and between

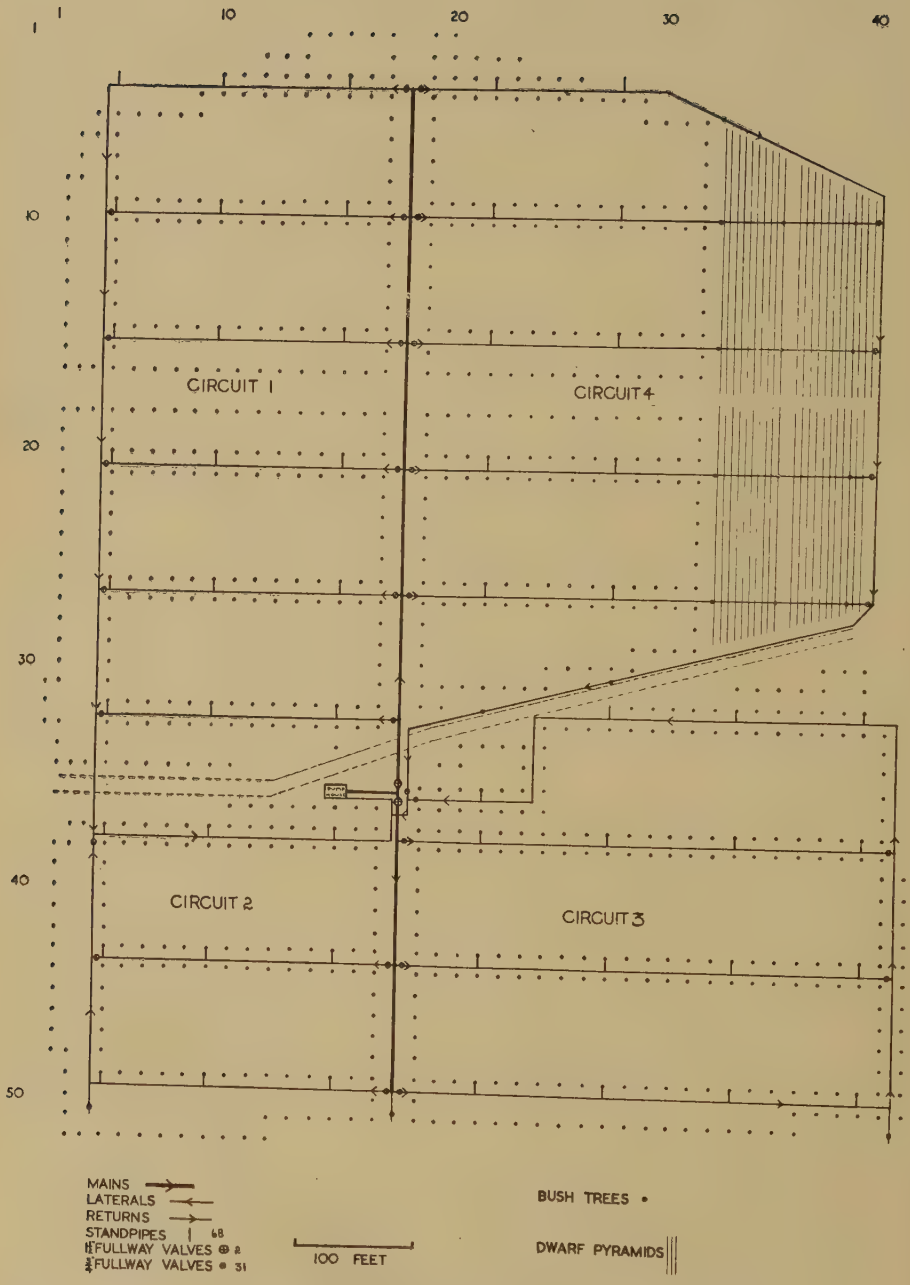


*Fig.* 2. Plan of Underground Spray Mains, Rivers Farm, Milverton.
(Bush trees shown in outline only)

standpipes permits the easy spraying from each stand pipe of a group of 36 bush trees 18 feet apart with a hose length of 90 feet.

This spacing of stand pipes is approximately $4\frac{1}{2}$ per acre and should be regarded as the minimum number. The greater the number the shorter the length of hose required, with considerable reduction in labour of hose pulling and moving by the sprayman.

(9) The siting of the stand pipes was modified to facilitate spraying the pyramid apples on the North East side of the orchard.

(10) Stand pipes are sited when convenient on the return circuit pipes. Stand pipes are on the North side of the laterals to permit of drainage.

The piping was installed in an established orchard and therefore by siting the laterals midway between rows of trees the least harm was caused to roots. The stand pipes were sited close to trees so that they caused the least possible obstruction to cultivating equipment and gang mowers. The trenches for the pipe were partly ploughed out and hand dug to a depth of 15—18 inches.

The pipe should be of Grade B galvanised and all the joints made with graphite paste packing and liberally painted externally with a good quality bitumen paint. In this particular lay-out red steam pipe was used through supply difficulties. It appeared from reports of the local soil conditions that this grade of steam pipe resisted corrosion for very long periods. In some soils the rate of external corrosion is so rapid that galvanised pipe has a very short life and it is necessary to use a bitumen wrapped pipe. In these circumstances it is wiser to avoid underground pipes.

**Drainage of the Pipe Circuits.**

Particular care was given to the easy and complete drainage of the various pipe circuits and equipment within the pump house. The lay-out of the plumbing was arranged so that the number of draining points was reduced to the minimum.

The drainage of the pipe circuits in the orchard was greatly facilitated by the sloping gradient of the land. The pump house is sited in a midway position on the slope and it is possible to drain the pipe circuits above the house into the lower circuits, thereby avoiding waste of wash. The complete drainage of the circuit is made possible by opening drainage valves at the bottom of the gradient below the last lateral.

The drainage of underground mains on a level site is sometimes troublesome. It is wise, during the laying down of the pipes in the trenches to make provision for drain valves at the lowest point. One stand pipe at the highest point must be left open to permit the entry of air ; in some cases it is better to drain sections of the orchard circuit separately.

## ACKNOWLEDGMENTS.

The described spray mains system was installed by A. Wimbush, Esq., Rivers Farm, Milverton, Somerset in 1946/47. Many thanks are due to Mr. Wimbush for permitting us to prepare the specification, to supervise the installation with local labour and for allowing us ready access to the pump house for making records.

## SUMMARY.

(1) An underground spray mains system suitable for 15—25 acres of top fruit is described.

(2) The system has a return circuit to the pump house which aims at maintaining constant and high-flow pressures at the lances.

(3) The return circuit system ensures that materials are maintained in suspension by the velocity of the fluid, thereby materially reducing the build-up of deposits.

(4) The lay-out of the pump house aims at labour saving, rapid filling of tanks and efficient agitation.

(5) The piping in the pump house is arranged so that records of pump and pipe circuit performances can be readily determined.

REFERENCE.

(1) Kearns, H. G. H. Hydraulic spraying machinery for fruit crops. The choice of power equipment. *Ann. Rept. Long Ashton Res. Sta.*, 1945, 110-132.

# THE QUALITATIVE EXAMINATION OF INSECTICIDAL PROPERTIES.

## PROGRESS REPORT, 1948.

By S. H. BENNETT, H. MARTIN, A. STRINGER and D. WOODCOCK.

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The discovery and introduction of new insecticidal compounds continues unabated but during the past year attention has been given only to those substances which seem of interest either because of their structural resemblance to known insecticides or because their mode of action appears to be different. The reason for the first choice is academic, for the information may help to explain why a particular molecular structure is toxic ; the reasons for the second choice involve the practical points that a range of compounds of diverse toxic action would reduce the risk that a continuous use of insecticides similar in action would lead to an unwanted selection of resistant strains of insect and that a mixture of two or more insecticides of diverse action is more economic than a single insecticide. For the latter reasons the systemic insecticides would seem of special importance but the poison hazards of the known compounds able to confer systemic insecticidal properties appear to be so dangerous that their practical use is out of the question until the extent of the risks are known with greater certainty.

As the methods employed have been described in previous reports, it is possible to proceed at once to indicate the results obtained.

### STOMACH POISONS.

#### A. Polychloroethane derivatives.

To obtain rough estimates of the toxicity of various compounds related in structure to DDT, cabbage leaf sandwiches were fed to third instar larvae of either *Mamestra brassicae* or *Pieris brassicae*. From the survivals it is possible to give the dose tolerated and in Table I this dose is recorded with the indication that the toxic dose is larger.

The preparation and characteristics of the compounds tested have been given in either the 1943 or the 1944 Progress Report ; in addition the following compounds, some of which were prepared by Dr. R. L. Wain, were included :—

*αα-bis-(4-chlorophenyl)-αββ-trichloroethane* : prepared by the addition of chlorine to *αα*-bis-(4-chlorophenyl)-*β*-chloroethylene crystallised from methyl

TABLE I.

DDT Analogues as Stomach Poisons

| Compound | Formula | Lethal dose (mg./g. body weight) to | |
|---|---|---|---|
| | | M. brassicae | P. brassicae |
| DDT | $(p\text{-Cl.C}_6\text{H}_4)_2\text{CH.CCl}_3$ | > 0·07 | < 0·01 |
| αα-bis(4-chlorophenyl)-ββ-dichloroethane | $(p\text{-Cl.C}_6\text{H}_4)_2\text{CH.CHCl}_2$ | — | *circa* 0·01 |
| αα-bis(4-chlorophenyl)-αββ-trichloroethane | $(p\text{-Cl. C}_6\text{H}_4)_2\text{C Cl.CHCl}_2$ | — | *circa* 0·1 |
| αα-bis(4-hydroxyphenyl)-βββ-trichloroethane | $(p\text{-HO.C}_6\text{H}_4)_2\text{CH.CCl}_3$ | — | > 6·7 |
| αα-bis(4-methylphenyl)-βββ-trichloroethane | $(p\text{-CH}_3\text{C}_6\text{H}_4)_2\text{CH.CCl}_3$ | — | *circa* 0·05 |
| αα-bis(4-methoxyphenyl)-βββ-trichloroethane | $(p\text{-CH}_3\text{O.C}_6\text{H}_4)_2\text{CH.CCl}_3$ | — | *circa* 0·05 |
| αα-bis(4-fluorophenyl)-βββ-trichloroethane | $(p\text{-F.C}_6\text{H}_4)_2\text{CH.CCl}_3$ | — | *circa* 0·05 |
| αα-bis(4-bromophenyl)-βββ-trichloroethane | $(p\text{-Br.C}_6\text{H}_4)_2\text{CH.CCl}_3$ | — | *circa* 0·02 |
| αα-bis(4-iodophenyl)-βββ-trichloroethane | $(p\text{-I.C}_6\text{H}_4)_2\text{CH.CCl}_3$ | — | *circa* 0·10 |
| αα-diphenyl-βββ-trichloroethane | $(\text{C}_6\text{H}_5)_2\text{CH.CCl}_3$ | — | *circa* 0·5 |
| αα-(4-*iso*propylphenyl)-βββ-trichloroethane | $(p\text{-Pr .C}_6\text{H}_4)_2\text{CH.CCl}_3$ | > 2·1 | *circa* 0·3 |
| α-α-bis(1-hydroxynaphthyl)-βββ-trichloroethane | $(1\text{-HO.C}_{10}\text{H}_6)_2\text{CH.CCl}_3$ | -- | > 5·0 |
| α-phenyl-α-(4-chlorophenyl) βββ-trichloroethane | $(p\text{-Cl.C}_6\text{H}_4)(\text{C}_6\text{H}_5)\text{CH.CCl}_3$ | — | *circa* 0·20 |
| α-(2-chlorophenyl)-α-(4-chlorophenyl)-βββ-trichloroethane | $(o\text{-Cl.C}_6\text{H}_4)(p\text{-Cl.C}_6\text{H}_4)\ \text{CH.C Cl}_3$ | — | > 0·36 |
| α-(4-methylphenyl)-α-(4-chlorophenyl)-βββ-trichloroethane | $(p\text{-CH}_3\text{C}_6\text{H}_4)(p\text{-Cl.C}_6\text{H}_4)\ \text{CH.C Cl}_3$ | > 0·26 | *circa* 0·3 |
| αα-bis(4-chlorophenyl)-αβββ-tetrachloroethane | $(p\text{-Cl.C}_6\text{H}_4)_2\text{C Cl.CCl}_3$ | — | > 1·0 |
| αα-bis(4-chlorophenyl) ethylene | $(p\text{-Cl.C}_6\text{H}_4)_2\text{C} = \text{CH}_2$ | — | > 3·5 |
| α-phenyl-α-(4-chlorophenyl) -ββ-dichloroethylene | $(p\text{-Cl.C}_6\text{H}_4)(\text{C}_6\text{H}_5)\text{C} = \text{CCl}_2$ | — | *circa* 0·13 |

alcohol in large tablets m.p. 45-7° C. (Found : C, 47·4 ; H, 2·7 ; Cl, 50·0. $C_4H_9Cl_5$ requires C, 47·4 ; H, 2·6 ; Cl, 50·0%) (c.f. Läuger, Martin and Müller, Helv. Chim. Acta., 1944, *27*, 914).

*αα-bis-(4-isopropylphenyl)-αββ-trichloroethane* : Prepared using cumene and chloral hydrate in a Baeyer condensation this compound was a viscous liquid b.p. 187-190° C./4 mm.

*α-(4-chlorophenyl)-α-(4-methylphenyl)-βββ-trichloroethane* : Prepared by the condensation of *βββ*-trichloro-*α*-(4-chlorophenyl)ethanol and toluene ; crystallised from methanol in slender needles m.p. 84-85° C. Chattaway and Muir (J.C.S., 1934, 701) give m.p. 81° C.

*αα-bis-(4-chlorophenyl)ethylene* : Prepared from 4:4′-dichlorobenzophenone crystallised from ethyl alcohol in prisms m.p. 86-87·5° C. (Found : C, 67·4 ; H, 3·9 ; Cl, 28·7. Calc. for $C_{14}H_{10}Cl_2$ : C, 67·5 ; H, 4·0 ; Cl, 28·5%). Bergmann and Bondi (Ber., 1931, *64*, 1455) give m.p. 91° C.

The results assembled in Table I confirm previous conclusion that (a) of substituents alternative to chlorine in the 4-position of DDT, fluorine, bromine and to a lesser degree iodine, the methyl, methoxy and to a lesser degree *iso*propyl groups, confer moderate insecticidal properties, (b) that asymmetry reduces insecticidal properties, and (c) that complete chlorination of the -CH.C $Cl_3$ group of DDT eliminates toxicity.

**B. Hexachlorocyclohexane.**

Further tests with the samples of the alpha-, beta- and gamma-isomers of benzene hexachloride, received by the courtesy of I.C.I. Ltd., are included in Table II.

TABLE II.

Isomers of Hexachlorocyclohexane as Stomach Poisons

| | Lethal dose (mg./g. body weight) to | |
|---|---|---|
| | M. brassicae | P brassicae |
| Alpha isomer (m.p. 154—5°) .. .. | > 0·13 | > 0·36 |
| Beta isomer (m.p. 297—9°) .. .. | > 0·18 | > 0·30 |
| Gamma isomer (m.p. 112—5°) .. .. | > 0·16 | *circa* 0·001 |

The greater potency of the gamma isomer is clearly revealed in the tests on P. brassicae but the comparative resistance of M. brassicae to this isomer is in line with earlier tests recorded in the 1944 Report.

**C. Miscellaneous chlorinated derivatives.**

Among compounds reputed to have been used for insecticidal purposes in Germany during the war, 4-chlorophenyl-chloromethyl sulphone seemed of interest. A sample of this compound of m.p. 118-9°, submitted by Messrs. Dickinson & Son, was examined. Of the other compounds tested, the following were prepared by Dr. R. L. Wain :—

*Chloralide* : as described by Otto (Ann., 1924, *239*, 262) crystallised from ethyl alcohol in colourless needles, m.p. 114-115° C.

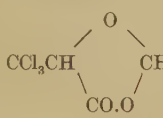
*Dichloralurea* : as prepared crystallised from aqueous alcohol in needles m.p. 194° C. (decomp.). Chattaway and James (Proc. Roy. Soc., 1932, A *134*, 372) give 194° C.

*γγγ-trichloro-α-nitro-β-hydroxypropane*: prepared according to the method of Chattaway and Witherington (J.C.S., 1935, 1178); rhombic plates, m.p. 45-46° C., from petroleum ether (b.p. 40-60°).

*α-nitro-βγγγ-tetrachloropropane*: prepared from the above hydroxy compound using thionyl chloride was a pale yellow oil b.p. 200-203°/764 mm. Henry (Rec. Trav. Chim. 1898, *17*, 1) gives b.p. 199-200° C./767 mm.

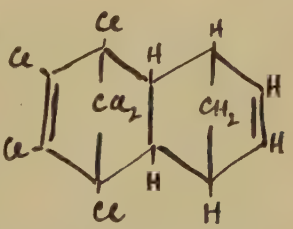
TABLE III.

MISCELLANEOUS CHLORINATED DERIVATIVES AS STOMACH POISONS

| Compound | Formula | Lethal dose (mg./g. body weight) to | |
|---|---|---|---|
| | | M. brassicae | P. brassicae |
| 4-chlorophenyl-chloromethyl sulphone | $p$-Cl.$C_6H_4SO_2.CH_2Cl$ | > 0·78 | > 0·26 |
| Bis(4-chlorophenyl) ketone | $(p\text{-Cl.}C_6H_4)_2CO$ | — | > 4·9 |
| α-(4-chlorophenyl)-βββ-trichloroethanol acetate | $(p\text{-Cl.}C_6H_4)CH(CCl_3)O.COCH_3$ | — | > 3·4 |
| Chloretone | $(CH_3)_2CH(OH)CCl_3$ | — | > 2·0 |
| Chloralide |  | — | > 3·0 |
| Dichloral urea | $(CCl_3CH(OH)NH)_2CO$ | — | > 4·4 |
| γγγtrichloro-α-nitro-β-hydroxypropane | $CCl_3CH(OH).CH_2NO_2$ | — | > 1·7 |
| α-Nitro-βγγγ-tetrachloro-propane | $CCl_3CHCl.CH_2NO_2$ | — | > 4·5 |
| 4:4′-dichloro tolane | $(p\text{-Cl.}C_6H_4C\equiv)_2$ | — | < 0·3 |
| αβ-di-(4-chlorophenyl)-αβ-dichloroethane | $p\text{-Cl.}C_6H_4CHCl.CHCl(p\text{-Cl.}C_6H_4)$ | — | > 3·5 |
| αβ-di-(4-chlorophenyl)-αβ-dibromoethane | $p\text{-Cl.}C_6H_4CHBr.CHBr.(p\text{-Cl.}C_6H_4)$ | — | > 4·5 |
| ααββ-tetrakis(4-bromo-phenyl) ethylene | $(p\text{-Br.}C_6H_4)_2C{=}C(p\text{-Br.}C_6H_4)_2$ | — | > 4·2 |

With the exception of 4:4′-dichlorotolane, all the compounds including 4-chlorophenyl-chloromethyl-sulphone were ineffective stomach poisons.

During his visit to America in the summer of 1948, Dr. R. A. E. Galley was given a sample (mp. 102·5-103° C.) of a new insecticide discovered in the laboratories of Julius Hyman and Co., where it was known as Compound 118, which was subsequently formulated as 1,2,3,4,10,10-hexachloro-1:4,5:8-diendomethano-1,4,4a,5,8,8a-hexahydronaphthalene.



This compound was fed to fourth instar larvae of *M. brassicae* and proved

toxic at a mean dose of 0·28 mg ± 0·006 per g. body weight. In parallel experiments the toxic dose of DDT was found to be 0·12 mg ±0·022 per gm.

**D. Rotenone.**

Läuger, Martin and Müller (1944) suggested that the toxophore of the rotenone molecule is the grouping—CO.CL : CL.OL, where L indicates other groupings which impart to the molecule the physical properties required to penetrate to the site of action of rotenone within the insect. As this toxophore is present in the condensation products of dimedone with aldehydes, one such compound was tested.

*Anhydride of dimedone—acetaldehyde derivative*: prepared by cyclisation of the dimedone—acetaldehyde condensation product with $N/_1HCl$, this crystallised from ethyl alcohol in colourless plates, m.p. 174-5° C.

The results given in Table IV show that the condensation product was non-toxic; the tolerance of rotenone by *M. brassicae* has already been noted in the 1943 Report.

TABLE IV.

ROTENONE AS STOMACH POISON

| | Lethal dose (mg./g. body weight) to | |
|---|---|---|
| | M. brassicae | P. brassicae |
| Rotenone .. .. .. .. | > 2·7 | < 0·6 |
| Dimedone derivative .. .. .. | > 3·5 | > 4·2 |

**E. Organophosphorus compounds.**

Bis(dimethylamino)fluorophosphine oxide, the systemic insecticidal properties of which are discussed later, was included in stomach poison tests alongside two derivatives, prepared by Messrs. Albright & Wilson Ltd., in which the fluorine was replaced, in one case by the 2-methyl-4,6-dinitrophenyl group, and in the second by 8-hydroxy quinoline. The former grouping is known to be highly insecticidal and finds use in DNC; the latter group has fungicidal properties.

Neither of these three compounds prevented the pupation of fourth instar larvae of *M. brassicae*, the amounts consumed being :—

Bis(dimethylamino)-2-methyl-4:6-dinitrophenoxy phosphine oxide: 1·40, 0·85, 0·28, 0·28, 0·41, 1·61 mg./g. body weight.

Bis(dimethylamino)-8-quinolyl-phosphine oxide: 0·59, 0·95, 1·10, 0·27 mg./g. body weight.

Bis(dimethylamino)fluorophosphine oxide: 0·48, 0·32, 0·54, 0·68, 0·68 mg./g. body weight.

The non-toxicity of the fluorophosphine oxide is surprising in view of the high degree of systemic toxicity imparted to treated plants, but the larvae were reluctant to take the treated sandwiches and much liquid vomit or excrement was observed.

## CONTACT INSECTICIDES.

The filter paper 'film' technique was used throughout with *Calandra granaria* as test insect. The compound under test was usually applied in known amount in a volatile solvent to the filter paper, on which, after evaporation of the solvent, a number of weevils were confined by a large glass ring.

**F. Chlorinated hydrocarbons.**

When deposited from acetone solution, DDT exhibited an extremely low increase in kill as the amount of DDT deposited is increased. For comparison with Compound 118 (see note C above) solutions in a non-volatile solvent (P.31 petroleum oil) were therefore used, 0·125 ml. of the solution being used per filter paper. The percentage kills of *C. granaria* (50 per paper), after the stated time, are given in Table V.

TABLE V.

DDT and Compound 118 as Contact Insecticides

| | | % kill at | | | |
|---|---|---|---|---|---|
| | Conc. mg./ml. | 68 | 90 | 120 | 140 hrs. |
| DDT P.31 oil | 5·00 | 76 | 98 | 100 | 100 |
| | 3·33 | 62 | 82 | 94 | 98 |
| | 2·22 | 42 | 66 | 74 | 84 |
| | 1·48 | 30 | 48 | 64 | 80 |
| | 0·99 | 24 | 40 | 52 | 72 |
| | 0·66 | 14 | 34 | 42 | 68 |
| | 0·44 | 8 | 26 | 32 | 50 |
| | 0·29 | 4 | 16 | 24 | 42 |
| 118 P.31 oil | 10·00 | 48 | 78 | 90 | 100 |
| | 6·67 | 34 | 62 | 84 | 96 |
| | 4·45 | 34 | 56 | 82 | 94 |
| | 2·96 | 30 | 54 | 80 | 92 |
| | 1·98 | 18 | 44 | 72 | 86 |
| | 1·32 | 16 | 42 | 64 | 80 |
| | 0·88 | 14 | 30 | 56 | 78 |
| | 0·59 | 4 | 18 | 44 | 62 |
| | 0·39 | 0 | 14 | 32 | 54 |
| | 0·26 | 0 | 10 | 16 | 44 |
| | 0·17 | 0 | 2 | 4 | 30 |
| | 0·12 | 0 | 1 | 2 | 20 |
| Control | — | 0 | 1 | 2 | 4 |

From the provisional probit-log concentration lines applied to these results it is apparent that the speeds of action of the two compounds differ widely and the relative magnitude of the median effective doses (DDT : Compound 118)

varies with time as follows :—after 68 hours, 4·5 : 1 ; after 90 hours, 2·5 : 1 ; after 120 hours, 1 : 1 and after 140 hours, 0·8 : 1.

Compound 118 is therefore as effective as DDT though slower in action, a difference indicative of a different mode of action. Compound 118 is also of interest for it is the first of a long series of chlorinated hydrocarbons, including DDT and its analogues, BHC, Toxaphene and Chlordane, to depart from the hypothesis that in such compounds ready dehydrochlorination is required for insecticidal properties (Martin and Wain, 1944). Compound 118 is not susceptible to ready dehydrochlorination.

Through the kindness of Monsieur M. Raucourt of the Laboratoire de Phytopharmacie of the French Ministry of Agriculture, samples of a mono-sulphurated and a thiocyanate derivative of hexachloro*cyclo*hexane were received. The former, usually referred to in the French literature as SPC (Sulfure de polycyclocyclane), is made by the reaction of benzene hexachloride with an alkali sulphide (Raucourt and Bégué, 1946) but the precise structure of the reaction product is still unknown ; in the latter one or more of the chlorines of benzene hexachloride is replaced by the thiocyanate group. The samples received were, however, far from pure and were not subjected to chemical check but their effect on *C. granaria* in oil deposits were compared with three samples of benzene hexachloride, the first the gamma isomer, the second a commercial BHC stated to contain 30% of the gamma isomer and the third the crude ether extract of a proprietary flea bettle dust which was used in the tests recorded in the 1944 Report.

From the results given in Table VI, provisional regression lines were drawn and those for the two French samples are parallel but of steeper slope than the lines given by the benzene hexachloride samples. Consequently a comparison on an ED 50 basis is impossible, but the difference in slope is evidence that the French samples contain insecticides of different action to benzene hexachloride. The thiocyano-derivative is less insecticidal than the sulphurated derivative, the ED 50 being 14·1 and 74·1 mg./ml. respectively. The corresponding figures for the BHC samples are 1·8 mg./ml. for the gamma isomer, 6·8 mg./ml. for the sample containing 30% gamma isomer and 33· mg./ml. for the extract.

TABLE VI.

Contact Toxicity of BHC Samples and Derivatives to *C. Granaria*.
(% *kill at* 24 *hours*).

| Conc. mg./ml. P31 | Mono-sulphurated] derivative | Thiocyano-derivative | Gamma isomer | BHC (30% gamma isomer) | Extract of Flea beetle dust |
|---|---|---|---|---|---|
| 50·0 | 68 | 44 | 76 | 70 | 60 |
| 25·0 | 62 | 34 | 70 | 58 | 46 |
| 12·5 | 52 | 22 | 62 | 50 | 40 |
| 6·3 | 34 | 18 | 60 | 48 | 32 |
| 3·1 | 22 | 8 | 58 | 46 | 30 |
| 1·6 | 14 | 2 | 46 | 38 | 20 |

**G. Aliphatic Thiocyanates.**

The practical utilization of *n*-dodecyl thiocyanate as aphicide or ovicide is frustrated by the high cost and unavailability of cocoanut oil, the natural

source of the dodecyl radical. An alternative source is from petroleum oil from which long chain primary alcohols may be produced by the Oxo process, but unfortunately this yields a mixture of isomers of which the primary alcohol probably amounts to 50%. The thiocyanates of the secondary alcohols are much less ovicidal than the primary alcohol thiocyanates (Bennett, Kearns and Martin, 1947). A sample of dodecyl thiocyanate from petroleum sources, supplied by Shell Chemicals Ltd., was therefore compared to "lauryl rhodanate" (the original commercial sample of *n*-dodecyl thiocyanate) by the filter paper method on *C. granaria*. From the results given in Table VII, provisional regression lines give the relative potency of "lauryl rhodanate" to be 2·03 that of the dodecyl thiocyanate.

TABLE VII.

DODECYL THIOCYANATES AS CONTACT INSECTICIDES

| mg./filter paper | % kill at 70 hours | |
|---|---|---|
| | "lauryl rhodanate" | Dodecyl thiocyanate |
| 5·00 | 100 | 100 |
| 2·50 | 100 | 100 |
| 1·25 | 100 | 80 |
| 0·65 | 82 | 70 |
| 0·31 | 61 | 48 |
| 0·16 | 50 | 34 |
| 0·08 | 44 | 30 |
| 0·04 | 32 | 20 |

**H. Organophosphorus Compounds.**

The extreme potency of *p*-nitrophenyl diethyl thiophosphate (Parathion, E 605) as a stomach poison is recorded in the 1947 Report. The testing of this compound as a contact insecticide by the "film technique" is complicated in some measure by its fumigant action but in the results to be given below, the time at which mortality counts were made is less than that taken for mortality in the "fumigant" tests. Fumigant action is therefore unlikely to influence the results of the qualitative test.

Commercial preparations of Parathion are likely to contain, in addition to the main ingredient, unreacted *p*-nitrophenol, triethyl thiophosphate and bis(*p*-nitrophenyl) ethyl thiophosphate. The phenol is known not to possess strong insecticidal properties but tests of the other two compounds were made on samples supplied by Messrs. Albright and Wilson. An attempted separation of crude Parathion into its components by distillation *in vacuo* after washing out unreacted phenol gave, however, unidentified fractions which contained variable amounts of sulphur easily oxidised by 55% nitric acid. The latter reagent was suggested by Schrader (1947) for the estimation of sulphur present in the thiono-form (i.e. doubly linked to phosphorus in contrast to the mercapto-form in which it is present in the group $\equiv PSC_2H_5$). These fractions were subjected to bioassay, particulars being as follows:—

*Triethyl thiophosphate* $(C_2H_5O)_3PS$: Sample No. R7323 supplied by Messrs.

Albright and Wilson Ltd., was a colourless liquid (Found : ≡P=S, as triethyl thiophosphate, 109·3%).

*Bis(p-nitrophenyl) ethyl thiophosphate* $[p\text{-}NO_2C_6H_4O]_2PS.OC_2H_5$ : Sample No. R 7395, also supplied by Messrs. Albright and Wilson Ltd., was a very pale yellow crystalline solid, m.p. 126-128° C. (Found : ≡P=S as ethyl bis(*p*-nitrophenyl) thiophosphate, 93·5, 93·2%).

*Fraction C* : recovered at 120-180° C./0·4 mm. from crude Parathion (Sample No. PP 288YX supplied by Messrs. Plant Protection Ltd.) in 29·0% yield (Found : Thionosulphur, in terms of *p*-nitrophenyl diethyl thiophosphate, 50·9, 51·5%).

*Fraction D* : recovered at 120-180° C./0·4 mm. from crude Parathion (Sample No. PP 288YX) in 52·3% yield (Found : Thionosulphur, in terms of *p*-nitrophenyl diethyl thiophosphate, 17·2, 17·5%).

*p-Nitrophenyl diethyl thiophosphate* : purified by rapid distillation *in vacuo* and supplied by Messrs. Albright & Wilson Ltd. (Sample No. R 7401). (Found : thiono-sulphur, in terms of *p*-nitrophenyl diethyl thiophosphate, 80·7, 81·3%).

*Parathion* : Sample No. PP 288YX. (Found : Thionosulphur, in terms of *p*-nitrophenyl diethyl thiophosphate, 104·5, 106·8%).

Filter paper tests of the above materials deposited from acetone solution and using *C. granaria* gave the results assembled in Table VIII. Triethyl thiophosphate, bis(*p*-nitrophenyl) ethyl thiophosphate, and sodium diethyl thiophosphate proved non-toxic at doses of 5 mg. per filter paper (7 cm. No. 1 Whatman). Provisional regression lines for the remaining samples gave the following estimated relative potencies :—

| | | | |
|---|---|---|---|
| *p*-nitrophenyl diethyl thiophosphate (purified) | 1·00 | — | — |
| Parathion (sample PP 288YX) .. .. .. | 0·83 | 1·00 | — |
| Fraction C .. .. .. .. .. | 0·17 | 0·21 | 1·00 |
| Fraction D .. .. .. .. .. | 0·05 | 0·06 | 0·31 |

TABLE VIII.

CONTACT ACTION OF PARATHION

| Dose mg./paper | % kill at 24 hours. | | | | % paralysis at 24 hours. | |
|---|---|---|---|---|---|---|
| | Purified | PP 288YX | Fraction C | Fraction D | Fraction C | Fraction D |
| 1·25 | 100 | 100 | 94 | 17 | 100 | 92 |
| 0·99 | 100 | 100 | 85 | 8 | 100 | 46 |
| 0·70 | 100 | 100 | 77 | 0 | 100 | 6 |
| 0·53 | 100 | 100 | 52 | 0 | 100 | 0 |
| 0·40 | 100 | 100 | 17 | 0 | 83 | 0 |
| 0·30 | 98 | 96 | 6 | 0 | 25 | 0 |
| 0·22 | 94 | 88 | 2 | 0 | 0 | 0 |
| 0·17 | 81 | 75 | 0 | 0 | 0 | 0 |

The potency of the Parathion sample relative to the purified sample is in agreement with expectations but samples C and D show an unexpected loss of

toxicity though their relative potencies (1.00 : 0·31) are in good agreement with their content of thiono-sulphur (51·2 : 17·3).

In a further investigation of the latter observation separate samples of the purified *p*-nitrophenyl diethyl thiophosphate were maintained at the distillation temperature (130-150° C.) for one, seven, and twenty-four hours, and another was exposed in a thin layer to ultra-violet light for 24 hours a check sample being held in the dark. Tests of the irradiated samples gave the figures of Table IX.

TABLE IX.

Contact Toxicity of Irradiated Parathion

| mg./filter paper | % dead after | | | |
|---|---|---|---|---|
| | 24 hours | | 48 hours | |
| | Irradiated | Check | Irradiated | Check |
| 5·00 | 100 | 100 | 100 | 100 |
| 3·33 | 100 | 96 | 100 | 100 |
| 2·22 | 0 | 0 | 68 | 74 |
| 1·48 | 0 | 0 | 0 | 0 |

The ultra-violet light treatment has not affected the potency of the *p*-nitrophenyl diethyl thiophosphate nor did it reduce the content of thiono-sulphur (80·7, 81·3% in dark ; 80·6% in irradiated sample).

The effects of heat treatment on the potency of *p*-nitrophenyl diethyl thiophosphate are shown in Table X.

The relative potency of the four samples is again highly correlated to their content of thiono-sulphur, the figures being :—

| | Relative potency | Thiono-sulphur (calc. to *p*-nitrophenyl diethyl thiophosphate |
|---|---|---|
| After 0 hours at 150° C. | 1·00 | 81·0 (1·00) |
| „ 1 „ „ „ | 0·95 | 75·8 (0·94) |
| „ 7 „ „ „ | 0·52 | 43·7 (0·54) |
| „ 24 „ „ 130-150° C. | Non-toxic | 0·0 (0·00) |

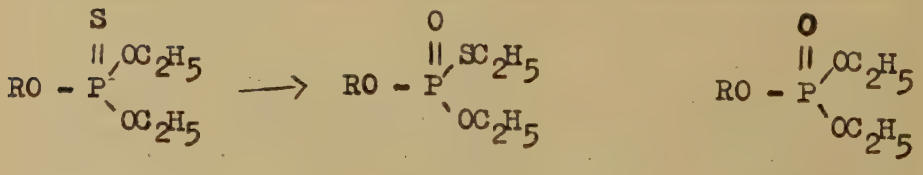
The computation of a single regression line on the basis of dose = mg. × thionosulphur content gave $\chi_9^2 = 13{\cdot}07$ and the results therefore substantiate the conclusion that insecticidal potency is correlated with thiono-sulphur content, estimated from the sulphuric acid produced by digestion with 55% nitric acid.

The nature of the change induced by heat treatment and of the crystalline derivative produced is under investigation. Schrader (1947) has stated that isomerisation of *p*-nitrophenyl diethyl thiophosphate (I, R = $p\text{NO}_2\text{C}_6\text{H}_4$—) to (II, R = $p$-$\text{NO}_2\text{C}_6\text{H}_4$—) begins at 130—150° C.

TABLE X.

EFFECT OF HEAT TREATMENT ON THE TOXICITY OF *p*-NITROPHENYL DIETHYL THIOPHOSPHATE

| No. of hours treated at 130—150° C. | % Thiono-sulphur (as nitrophenyl diethyl thiophosphate) | Dosage mg./paper | % kill at 24 hours |
|---|---|---|---|
| 24 | 0 | 5·00 | 2·5 |
| | | 2·50 | 0 |
| | | 1·25 | 0 |
| | | 0·63 | 0 |
| | | 0·31 | 0 |
| 7 | 43·7 | 2·50 | 100 |
| | | 1·25 | 100 |
| | | 0·63 | 97·5 |
| | | 0·31 | 55·0 |
| | | 0·16 | 10 |
| 1 | 75·8 | 0·71 | 100 |
| | | 0·36 | 95 |
| | | 0·18 | 80 |
| | | 0·09 | 15 |
| | | 0·05 | 0 |
| 0 | 81·0 | 0·50 | 100 |
| | | 0·25 | 92·5 |
| | | 0·13 | 55 |
| | | 0·06 | 2·5 |
| | | 0·03 | 0 |



I II III

If, however, isomerisation is the cause of the loss of insecticidal properties of I on heat treatment, the non-toxicity of II is unexpected in view of the known high potency of *p*-nitrophenyl diethyl phosphate (III, R = *p*-$NO_2C_6H_4$—).

## SYSTEMIC INSECTICIDAL ACTION.

The ability of plants to take up bis(β-fluoroethoxy)methane and to become systemically insecticidal, reported last year, has now been confirmed with two other compounds, bis(dimethylamino)fluorophosphine oxide, $[(CH_3)_2N]_2POF$ and bis(bisdimethylamino)phosphonous anhydride $[(CH_3)_2N]_2PO.O.PO[N(CH_3)_2]_2$. A sample of this fluorophosphine oxide was kindly supplied by Messrs. Albright and Wilson Ltd., but of the latter compound the only sample available was a commercial product, Pestox III, estimated to contain about 60% anhydride.

Further tests on the possibility of fumigant action from soil watered with dilute solutions of these compounds were made with potted cauliflower plants infected by the Mealy Cabbage aphis (*Brevicoryne brassicae* (L.)). It was found that if the insecticide was applied to filter paper roughly equivalent in area to the leaf span of the plant and spread around the stem of the plant at soil level, many of the aphides fell from the plant though a 100% control was not obtained. The fumigant action of the phosphonous anhydride is considerably less than either the fluorophosphine oxide or the fluoroethoxy methane. On the other hand, if the same amount of insecticide in water solution was applied to the soil which was then covered with waxed paper sealed with wax at the pot and the stem of the plant, the plants were completely cleared of aphides in 3—7 days. Treatment of the soil at 40 p.p.m. with either the fluorophosphine oxide, the fluoroethoxymethane or the phosphonous anhydride cleared the cauliflower of aphides and at 200 p.p.m. they were kept clean for 4—5 weeks.

To eliminate the possibility of fumigant effects, experiments were continued on willow sets about 6—8 in. long which, in aqueous nutrient solution, had rooted and thrown a main shoot 18—24 ins. long. The set was transferred to an aqueous solution of the insecticide and removed back to nutrient solution after it had taken up a known amount of insecticide. The possibility of a preferential absorption of insecticide was checked by a rough quantitative comparison of the fumigant action of the insecticide solution after removal of the set and the original solution ; no differences in toxicity to adults of the Brassy Willow Beetle (*Phyllodecta vitellinae L.*) were found. For the examination of the toxic action of foliage from the treated willow sets, the same test insect was used.

Direct fumigant action of the aqueous solution of the insecticide against this insect was examined by placing 5 ml. of aqueous solution in the lower half of a 9 cm. Petri dish over which a muslin circle was stretched. The beetles were placed on this muslin and covered by the upper half of the dish. Solution containing only 0·5 mg. of either the fluorophosphine oxide or the fluoroethoxy methane showed fumigant action against *P. vitellinae* L. under this condition, the former being more effective and rapid in action than the latter. Under like condition the phosphonous anhydride showed no action.

Beetles placed with leaves picked from treated sets were quickly killed, yet the amount of feeding by the beetles was extremely small. In such tests it is evident that death may follow from fumigant, stomach poison or contact insecticidal action ; toxicity found in such tests is accordingly called the " overall " toxicity.

In preliminary experiments to determine the mechanics of the absorption and translocation of the insecticide, it was noted that, under conditions of rapid transpiration, the leaves at the tip of a 24″ shoot became insecticidal within 4 hours of the set being placed in the insecticide solution. If, however,

a leaf near the tip of the shoot is placed in a 3 × 1″ tube and sealed around the petiole with vaseline prior to the roots being immersed in the insecticidal solution, it does not become insecticidal though leaves above and below the sealed leaf, removed 8 hours after treatment, were rapidly toxic to the beetle. The fact that the tubed leaf is held in a saturated atmosphere by the time the insecticide reaches that part of the plant, indicates that the insecticide moves in the transpiration stream and that it failed to reach leaves from which transpiration was arrested by non-evaporation from the leaf surface.

Attempts were made to assess the distribution of the insecticide in the willow set by a comparison of the "overall" toxicity of separate parts of a set.

TABLE XI.

"Overall" Toxicity of Treated Willow Tissue to Phyllodecta Vitellinae

| | First symptoms after hours | 100% moribund after hours |
|---|---|---|
| Leaf .. .. | 1 | 5 |
| Shoot .. .. | 1 | 24 |
| Stem .. .. | 2 | 40 |
| Solution .. .. | 16 | 65 |

A set was treated with 0·5 ml. of a 1·0% solution of the fluorophosphine oxide per gm. weight of set. 0·1 gm. of tissue was taken and the effect of the leaves, shoot and stem are shown in Table XI. The leaf is more toxic than the shoot which in turn is more toxic than the stem ; all are more toxic than the fumigant action of the solution containing the amount of insecticide estimated to be present in the plant tissue, assuming an improbable even distribution of the insecticide throughout the set.

The latter observation, suggesting that toxicity is dependent on the total amount of water present through its effect on phase distribution of the insecticide, prompted further trials (Table XII) in which leaves from treated

TABLE XII.

Toxicity of Willow Leaf Tissue to Phyllodecta Vitellinae

| | First symptoms after hours | 100% moribund after hours |
|---|---|---|
| "Overall" toxicity : normal humidity .. .. | 1 | 5 |
| „ „ high humidity .. .. | 2 | 6 |
| Fumigant action : shredded leaf : normal humidity .. | 3 | 6 |
| „ „ „ „ placed in water .. | 4 | 48 |
| „ „ whole leaf : normal humidity .. | 1 | 12 |
| „ „ „ „ placed in water .. | 1 | 64 |
| Solution : 0·0005 g. .. .. .. .. | 16 | 65 |
| „ 0·05 g. .. .. .. .. | 1 | 4 |

sets were tested in the presence of excess water. The shredded leaf was more toxic than the whole leaf, as might be expected for the surface area of treated tissue exposed to air is greater when the leaf is shredded. But, on the estimate that the leaf tissue used contained 0·0005 g. of the fluorophosphine oxide, the leaf is about 100 times as effective as the solution containing this amount. These results suggest that the fluorophosphine oxide acts as a water-soluble fumigant and that the effective vapour phase concentration is dependent on the concentration of the insecticide in the aqueous phase.

Using Lepidopterous larvae as test insects it was found that aqueous 1·0% solution containing 50 mg. of either the fluorophosphine oxide or the phosphonous anhydride were ineffective as fumigants against last-instar larvae of *P. brassicae*, but using 1·0% solution containing 50 mg. of the fluoroethoxymethane, the larvae were moribund within 4 hours. Again with larvae of the tomato moth (*Diataraxia oleracea* L.) only the fluoroethoxy methane was an effective fumigant in 1·0% aqueous solution at 50 mg. insecticide. But using leaves from cauliflower plants which absorbed 5 mg./gm. of tissue of the fluorophosphine oxide effective fumigant action and " overall " toxicity were shown ; leaves from plants treated with equivalent amounts of the fluoroethoxy methane, on the other hand, were ineffective in fumigant action but the *Diataraxia* larvae were generally repelled from the leaf with some deaths though only small amounts of leaf tissue were eaten. The phosphonous anhydride proved ineffective in all trials in which willow beetles or the lepidopterous larvae were used.

## SUMMARY.

1. Of sixteen DDT analogues tested as stomach poisons against larvae of *Pieris brassicae* activity was shown only in symmetrical derivatives of which the nuclear chlorine of DDT was replaced by fluorine, bromine and, to a lesser degree, by iodine, or by the methyl, methoxy and, to a lesser degree, by isopropyl groups. Complete chlorination of the —$CH.CCl_3$ group eliminated toxicity.

2. The alpha-, beta- and gamma-isomers of hexachloro*cyclo*hexane were ineffective as stomach poisons to larvae of *Mamestra brassicae* and only the gamma isomer was toxic to larvae of *P. brassicae*. Samples of a sulphurated- and a thiocyano-derivative of hexachloro*cyclo*hexane gave probit-dosage regression lines of slope different to that given by hexachloro*cyclo*hexane in " film " contact tests using *Calandra granaria*.

3. Of twelve chlorinated derivatives tested as stomach poisons against larvae of *P. brassicae*, only 4:4′-dichlorotolane showed a moderate toxicity.

4. " Compound 118," a chlorinated naphthalene, showed a high stomach poison action to larvae of *M. brassicae* and was as toxic as, though slower in action than, DDT as a contact poison against *C. granaria*.

5. A dimedone-acetaldehyde condensation product proved inactive against larvae of *M. brassicae* and *P. brassicae*.

6. Dodecyl thiocyanate prepared from dodecyl alcohol from petroleum sources proved about half as toxic as *n*-dodecyl thiocyanate prepared from cocoanut oil when tested as contact insecticide against *C. granaria*.

7. Bis(dimethylamino)fluorophosphine oxide and two of its derivatives in which the fluorine atom was replaced by the 2-methyl-4:6-dinitrophenoxy group and by the 8-quinolyl group were ineffective as stomach poisons against larvae of *M. brassicae*.

8. Heat treatment of *p*-nitrophenyl diethyl thiophosphate at 130—

150° C. for 24 hours destroyed its toxicity as contact insecticide to *C. granaria*, the loss of toxicity being correlated with a reduction of $HNO_3$-oxidisable sulphur in the product. Exposure in ultra-violet light for 24 hours did not affect the toxicity nor the content of $HNO_3$-oxidisable sulphur in *p*-nitrophenyl diethyl thiophosphate.

9. Triethyl thiophosphate, bis(*p*-nitrophenyl)ethyl thiophosphate and sodium diethyl thiophosphate were ineffective as contact insecticides against *C. granaria*.

10. Both bis(dimethylamino)fluorophosphine oxide and bis(bisdimethylamino)phosphonous anhydride are capable of absorption by plants which become systemically insecticidal, though the latter was effective only against aphides. Both compounds and bis(betafluoroethoxy)methane have a fumigant action, as has the tissue from plants which have absorbed these compounds from aqueous solution.

11. Preliminary experiments indicate that the systemic insecticides function as water-soluble fumigants and move in the transpiration stream.

## ACKNOWLEDGMENTS.

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# NEW FUNGICIDAL SPRAYS FOR APPLES. *

By R. W. MARSH.

Many new fungicides have been developed in the past decade, mostly in the United States. The preliminary laboratory and greenhouse tests of fungicides, made under strictly controlled conditions, give results that are equally valid on both sides of the Atlantic. Thus we can start on this side with a knowledge of the *fungicidal value* of these materials : information that relieves us of the necessity of making these preliminary tests. But when you wish to know the *field performance* of a fungicide (a much broader conception than that of fungicidal value) then the American data are not necessarily applicable to Britain, as our conditions, especially in fruit growing, are radically different from those overseas.

It is sometimes assumed that British fruit growing is of too little importance in the world to merit special consideration. On that point, I will simply state the fact that the apple acreage of the United Kingdom is 30 per cent greater than that of Canada, twice that of Australia or South Africa ; twelve times that of New Zealand. I need hardly say that in our climate apple scab flourishes and I am of the opinion that more money is spent in spraying against apple scab than in spraying against any other fungus disease in this country.

**Comparison of home with overseas spraying programmes.**

How does the programme of fungicide applications on apples in Britain differ from that overseas ? In almost all other apple growing countries, codlin moth is a major pest. The pattern of the spray programme is decided by the necessity of making repeated summer applications of an arsenical or its equivalent. The fungicide thus becomes an adjunct to be mixed with lead arsenate, lime or oil (or all three) and applied frequently from June to September. The sequel is, of course, that washing of the fruit is accepted as a necessity.

In the United Kingdom, however, codlin is not a major pest in commercial apple plantations, and spraying specifically against codlin is unusual. Further, the apple grower is commonly not only a grower of apples but has plantations of plums and black currants as well. It therefore benefits him to be able to complete his apple spraying by the end of June. By then, new leaf production on apples is well past its peak and the sources of scab infections on the year-old shoots have dried up. Provided that the earlier sprayings have been effective, a spray material with high tenacity and lasting fungicidal value applied in late June has a reasonable chance of protecting the fruit and most of the foliage for the rest of the summer.

Hitherto, lime sulphur has been the fungicide chiefly used for this purpose and I have no doubt that it will remain in general use here for many years to come. It is cheap, tenacious, highly fungicidal and also acaricidal. Its disadvantages are that it is bulky, corrosive, unpleasant to use, often damaging and not compatible with oils. Recently, too, the supply has been inadequate, but presumably that is simply a reflexion of the present intensified demand for lime for building purposes.

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**Possible substitutes for lime sulphur.**

The possible substitutes for lime sulphur may be roughly grouped into four classes :

First, the dithiocarbamates, including the materials known commercially as Fermate, Zincate and Dithane. Included in this group is tetramethylthiuram disulphide, which has a wide variety of trade names such as Pomasol, Thiosan, Tersan and Jap beetle spray.

Second are the organic mercurials of which phenyl mercury chloride has been mainly investigated in England and the material known as Puratized N5 in U.S.A.

Third are the substituted quinones and quinolines which you have probably heard of under such names as Spergon, Phygon and Quinolate.

Fourth are the glyoxalidine derivatives usually listed under the term Fungicide 341.

As the results of trials made with these materials have recently been published (*J. Pomol. hort. Sci.*, 23, 185), I do not propose to do more than give you the results in a very summary form.

The black tenacious residue left by Fermate makes its use on apples impracticable unless the fruit is washed before sale. Tetramethylthiuram disulphide has been under trial at East Malling for many years and has given somewhat erratic results. I should tentatively class both these materials as mild fungicides, comparable to the wettable sulphurs, and needing repeated applications to be of value.

Phenyl mercury chloride has an exceptional fungicidal value and in general has not proved phytocidal, although certain experimental preparations have caused damage. There is a natural hesitation to adopt this, or other mercurials, until a permitted tolerance has been declared.

Preliminary tests with glyoxalidine in the dry season of 1947 were very promising, so the material was given a more stringent trial in the wetter summer of 1948. It was applied at a strength of 1 qt. per 100 gal. to trees of the variety Worcester Pearmain on 13 April, 23 April, 15 May and 10 June. Comparable trees were sprayed on the same dates with lime sulphur at 3, 2, 1 and 1% successively. Scab infection on the leaves was measured at the end of July and on the fruits at the end of August, giving the following results :

| *Treatment* | *Mean percentage surface area scabbed* | |
|---|---|---|
| | *per leaf* | *per fruit* |
| Lime sulphur .. .. .. | 0·602 | 0·106 |
| Glyoxalidine .. .. .. .. | 0·116 | 0·058 |
| No spray .. .. .. .. | 1·112 | 0·309 |

The amount of scab infection on the glyoxalidine-sprayed leaves was therefore only one-tenth of that on the control leaves and the infection on the fruits only one-sixth. On the leaves the reduction of infection significantly exceeded that given by the normal lime sulphur treatment.

One disadvantage of glyoxalidine, that it shares with all the newer fungicides mentioned, is that it does not control red spider, but small-scale tests indicate that glyoxalidine may be added to a derris-oil spray without increasing the risk of spray damage.

Hydroxyquinoline sulphate showed no fungicidal value in the apple scab control experiments made at Long Ashton in 1947.

**Spray residue problems.**

A brief mention must be made of the importance of investigating the effects of some of the newer fungicides when present as spray residues on processed fruit. On black currants, for example, the presence of any of the thiocarbamate sprays such as Fermate, Dithane, or tetramethylthiuram disulphide may be very undesirable. As little as 1 part per million of these materials may accelerate corrosion of the can and impair the flavour and appearance of the contents. As processing of fruits becomes more widespread the study of this particular aspect of spray residue effects is likely to gain in importance.

# A PROGRESS REPORT ON THE EFFECT OF ORGANIC CHEMICALS ON THE HEALING OF WOUNDS ON APPLE TREES.

By S. H. CROWDY.

## INTRODUCTION.

Earlier trials on the treatment of cankers on apple (caused by *Nectria galligena* Bres.) indicated that it might be possible to check the spread of cankers by treating them with compounds which would either stimulate the regeneration of the host tissues, or check the development of the pathogen without causing serious damage to the host (1). In view of this conclusion the present experiments were started to measure the activity of these compounds in stimulating the promotion of callusing to cover wounds. As in the previous trials, the substances were applied in lanoline. Lanoline is widely used as a vehicle for plant growth substances, and has also been described as a successful wound dressing (5). Up to the present there appears to have been little attempt to speed wound healing with plant growth substances though Shear (5) made some trials with 0·5% indoleacetic acid in lanoline, which he found less effective than the lanoline alone.

## METHOD.

The present experiments are in general similar to those carried out by Marshall (3) : holes were bored each month in the trunks and larger branches of the trees with a bit 1 in. in diameter. The depth of the holes was such that the cylinder of tissue removed included the youngest and most active wood cells. In this way it was hoped to reproduce more accurately the behaviour of cankers where the exposed wood tissues have been killed. The outline of the exposed wood surface in these holes was traced, at intervals during the year, on celluloid and the area of exposed wood was measured with a planimeter. It was also possible by making a further tracing of these celluloid plans to keep a permanent record of the progress of the experiment. In theory the initial area of exposed wood at each treatment should be about 5 sq. cm. but it was found in practice that there was some shrinkage of the surrounding peripheral tissue and the average initial area was more usually in the neighbourhood of 6 sq. cm. In some cases also there was splitting

of the tissues outside the wood, but it was assumed that variations due to this cause were part of the unavoidable errors associated with the method, and no allowance was made for them in the final analysis. In order to avoid possible complications due to the translocation of the substances in the trees, one compound only was used on each tree. The trees were all of the variety Lane's Prince Albert.

The following nine materials were included in the trial, and each was replicated five times :

1% γ-indolyl,3-butyric acid
1% bis(2-naphthoxy)acetic acid (1)
1% 4-chloro, 3-5-dimethylphenoxyacetic acid (3)
1% 2-4-6-trichlorophenoxyacetic acid (1)
10% 2-4-6-trichlorophenoxyacetic acid (1)
1% Cryptonol (8-hydroxyquinoline sulphate) (2)
10% Cryptonol (2)
All these were applied in lanoline.
Control, lanoline alone.
Control, untreated.

(1) supplied by Pal Chemicals Ltd.
(2) ,, ,, British Quinoline Ltd.
(3) ,, ,, Dickinson & Son Ltd.

Of these the first three were expected to stimulate wound healing ; 2-4-6-trichlorophenoxyacetic acid appeared, if anything, to reduce callus formation but was included because it had been found to be more toxic than the others to *N. galligena* in culture, while Cryptonol, although it had no growth stimulating properties and had even appeared phytotoxic at the higher concentration, seemed to be sufficiently toxic to the pathogen to merit inclusion in the trial. Neither 2-4-6-trichlorophenoxyacetic acid nor Cryptonol was soluble at a concentration of 10% in lanoline, and both were applied as suspensions, so that in these treatments the actual concentration of dissolved material available to the wound may have been considerably less than 10%.

The lanoline pastes were kept in lengths of glass tubing $\frac{3}{8}$-inch in diameter and graduated in centimetres : 2 cms. of paste were extruded on to each wound for each treatment. The average weight of 1 cm. of lanoline extruded in this way was 220 ± 20 mgm., and from this it was possible to calculate the average dosage of active principle applied, which in the case of the 1% pastes was 4·4 mgm.

The first series of wounds was made early in March and a fresh series was added to the trial at the beginning of each of the following months.

## EXPERIMENTAL RESULTS.

When considering the behaviour of wounds cut in this way there are two types which have to be distinguished, those in which only the surface tissues are damaged and the underlying wood is healthy and active, and those in which the underlying wood is damaged also. When the wood tissues are undamaged the live wood cells, especially those at the end of the medullary rays, take an active part in laying down the initial callus pad (4) so the behaviour of these wounds differs fundamentally from those in which the underlying wood is damaged and all the healing depends on the spread of callus from the edges of the wound.

In this experiment the active layers of wood were removed and healing

was from the edges. The general pattern of callus growth is shown in Figure 1, which is a reproduction of the plans made in September of the May wounds.

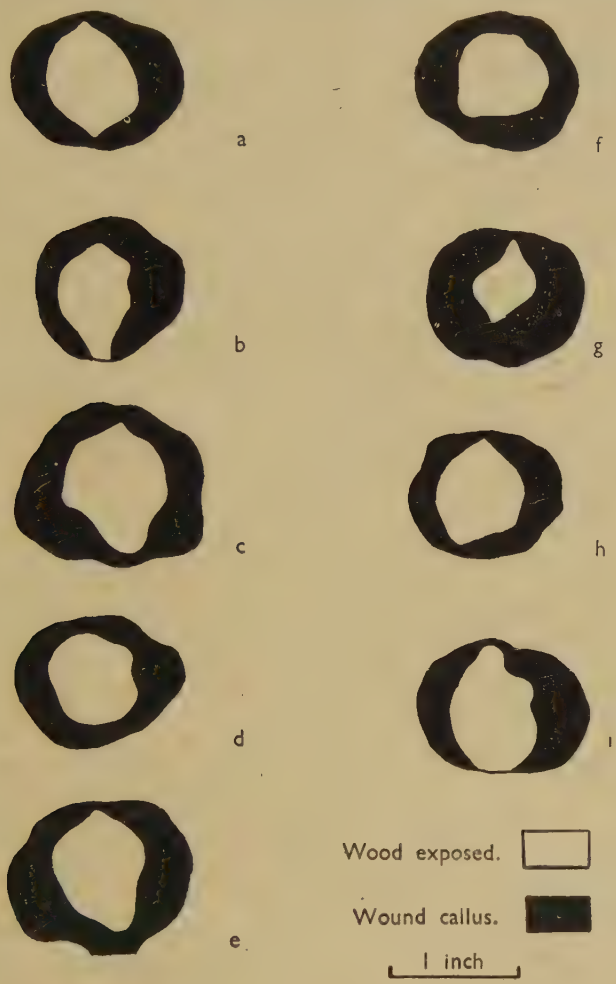



*Fig.* 1.

September. Callus diagrams of wounds made in May: *a*, Control untreated: *b*, Control lanoline: *c*, 2-4-6-Trichlorophenoxyacetic acid 10%: *d*, 2-4-6-Trichlorophenoxyacetic acid 1%: *e*, bis(2 naphthoxy)acetic acid 1%: *f*, 4-chloro, 3-5-dimethylphenoxyacetic acid 1%: *g*, γ-indolyl,3-butyric acid 1%: *h*, Cryptonol 1%: *i*, Cryptonol 10%.

The plans chosen for reproduction were those in which the area of exposed wood approximated most closely to treatment means shown in Table 1. The black area represents the callus tissue and the central unshaded patch the exposed wood. The outside edge of the plan shows the boundary of the wound in the bark. The diagrams are orientated as though the branch was running from the bottom of the page to the top, and it will be noted that there is a marked tendency for callusing to be stronger at the sides of the wound than at the top and bottom. The diameter of the wound in the bark is considerably more than that of the original wound and this can be attributed to two main

causes ; in the treatments in which callusing is active the growth of the callus pad tends to push aside the overlying tissues, thus apparently enlarging the hole, while in the phytotoxic treatments such as 10% 2-4-6-trichlorophenoxyacetic acid the enlargement is also due to the death and shrinkage of the tissues round the wound.

The general behaviour of the wounds during the period under trial is shown in figure 2. There is little difference in the behaviour of the wounds made in March, April and May, which suggests that regeneration does not start until May, and it appears that the growth of the callus practically ceases in September in the earlier wounds, but the period of callus production seems to be somewhat prolonged for the wounds made in July and August. The wounds made after August showed no signs of activity by the end of November. Chemical treatment does not appear to extend this active period.

In order to assess the value of the individual treatments a comparison was made within the series of wounds made in May. This series was chosen because the wounds had had the whole active period in which to heal and had also been exposed for a minimum time before healing began. The results of this comparison are shown in Table 1.

TABLE I.

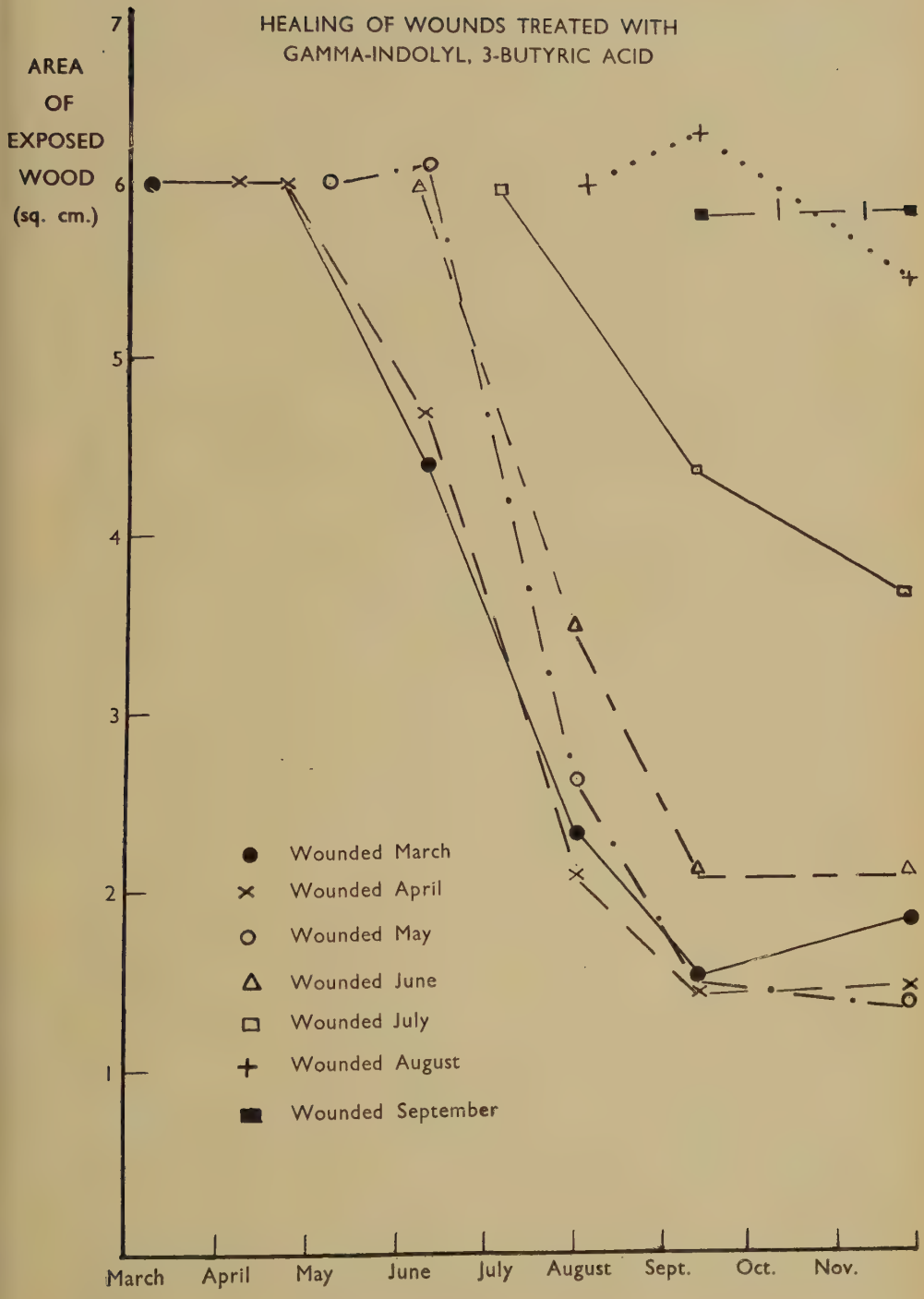
Recovery of Wounds Made at the Beginning of May.
(Initial area of exposed wood approx. 6 sq. cm.).

| Treatment | Area wood exposed : sq. cms. | |
|---|---|---|
| | 29.7.48 | 23.9.48 |
| Control, untreated .. .. .. .. | 4·5† | 3·2† |
| Control, lanoline .. .. .. .. | 3·8 | 2·4 |
| 2–4–6–trichlorophenoxyacetic acid 10% .. | 5·3† | 4·1† |
| 2–4–6–trichlorophenoxyacetic acid 1% .. .. | 4·2* | 2·6 |
| Bis(2·naphthoxy)acetic acid 1% .. .. | 4·6† | 3·3† |
| 4–chloro,3–5–dimethylphenoxyacetic acid 1% .. | 3·2† | 2·3 |
| γ-indolyl,3-butyric acid 1% .. .. .. | 2·6† | 1·5† |
| Cryptonol 1% .. .. .. .. | 4·1 | 2·8* |
| Cryptonol 10% .. .. .. .. | 4·4† | 3·2† |

* Differ from lanoline treatment significantly ($P = 0·05$).
† Differ from lanoline treatment very significantly ($P = 0·01$).

| | | | | |
|---|---|---|---|---|
| 29.7.48 readings : | difference in excess of | 0·33 | significant at | $P = 0·05$ |
| | ,, ,, | 0·44 | ,, | $P = 0·01$ |
| 23.9.48 readings : | ,, ,, | 0·37 | ,, | $P = 0·05$ |
| | ,, ,, | 0·49 | ,, | $P = 0·01$ |

It will be seen from this table that treatment with lanoline gave a marked improvement over the untreated wounds ; this is in agreement with Shear's findings (5). Since all the other treatments were applied in lanoline they must be compared with the lanoline-only treatment as a control. Healing was markedly poor in the wounds treated with 10% Cryptonol and 10% 2-4-6-trichlorophenoxyacetic acid : in these cases the tissues adjacent to the wounds were killed quite rapidly. The behaviour of these compounds could


HEALING OF WOUNDS TREATED WITH
GAMMA-INDOLYL, 3-BUTYRIC ACID
AREA
OF
EXPOSED
WOOD
(sq. cm.)
7
6
5
4
3
2
1
Wounded March
Wounded April
Wounded May
Wounded June
Wounded July
Wounded August
Wounded September
March
April
May
June
July
August
Sept.
Oct.
Nov.

have been inferred from the previous trial. It was surprising, however, that a marked reduction in regeneration also followed treatment with bis(2-naphthoxy) acetic acid, since it had been expected that this compound would stimulate healing. On the other hand, γ-indolyl,3-butyric acid produced a marked stimulation in healing and at all stages was very much the most effective of the treatments used ; this also was expected from the previous trial. 4-chloro,3-5 -dimethylphenoxyacetic acid produced a definite stimulation in the early stages, but this was not maintained throughout the season, the final area of exposed wood in the wounds being almost equal to that exposed in the wounds treated with lanoline alone. The remaining treatments differed little from the lanoline series : 1% 2-4-6-trichlorophenoxyacetic acid appeared to check healing to some extent in the early stages, but this check was not maintained, while the healing of the 1% Cryptonol series was slightly worse than the lanoline controls at the end of the season ; in both cases the differences were only just significant at the 5% level.

In addition to the main experiment described above a preliminary trial with 1% 2-4-dichlorophenoxyacetic acid was carried out. Exactly the same technique was used but the observations were only replicated twice. 2-4-dichlorophenoxyacetic acid is well known as an active growth stimulant, and it was felt that its behaviour as a wound dressing would be of interest. It was obvious at an early stage that the concentration used was very much too high ; the tissue surrounding the wounds was killed but beyond the dead zone there was marked swelling under the bark ; many of the flowers on the spurs near the wounds were retained throughout the season ; there was also a tendency for the leaves on neighbouring shoots to be twisted. The average area of the wood exposed at the end of the season, in the May wounds, was 5·3 sq. cms. The reaction of the trees to this treatment showed that the compound was capable of causing violent activity in the peripheral tissues, and it seems possible that it might be of value as a wound dressing if it were used at very much lower concentrations, though even then it would have to be used with care. In this connection it should be noted that in a recent attempt to eradicate poison ivy with a 2-4-dichlorophenoxyacetic acid spray there was a marked residual effect on certain varieties of apple, and the conclusion was reached that the cumulative effect of 2-4-dichlorophenoxyacetic acid over a period of years might result in tree injury (2).

## DISCUSSION.

Two main points of interest appear to have emerged from the present series of trials. In the first place the conclusion reached in the previous report (1), that the compounds which checked the spread of *N. galligena* cankers were also those which were able to stimulate healing, has to a large extent been confirmed. The behaviour of bis(2-naphthoxy)acetic acid provides a striking exception to this, but this result may be due to an excessive dose. In the second place there is an indication that the inclusion of plant growth substances in wound dressings may be a definite advantage, and that it may be possible to chose dressings which actively encourage healing instead of aiming only for a low phytotoxicity.

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# EXPERIMENTS ON THE CONTROL OF BROWN ROT OF FRUITS: PROGRESS REPORT, 1947-48.

By R. J. W. BYRDE.
(Department of Horticulture, University of Reading.)*

## SUMMARY.

Winter application of a phenyl mercury chloride preparation or of a calcium arsenite mixture inhibited spore formation on mummified apples and plums infected with *Sclerotinia fructigena*. Preparations of copper naphthenate showed no such fungicidal effect.

A late June spray of another phenyl mercury chloride preparation resulted in an approximately three-quarters reduction in the current season's brown rot infection of plums in a small-scale trial; lime-sulphur showed a lesser degree of control.

## INTRODUCTION.

In this country, past attempts to control the brown rot diseases of fruit (caused by *Sclerotinia fructigena* Ader. and Ruhl. and *Sclerotinia laxa* Ader. and Ruhl.) by direct spraying have met with little success. Promising results have been obtained recently, however, by Marsh (1947), in a preliminary trial, using a phenyl mercury chloride preparation as an eradicant winter spray against brown rot of plum.

Earlier work involving the use of eradicant fungicides against the brown rot diseases was carried out with success in the U.S.A. by Wilson (1942), using sodium, zinc and calcium arsenites as winter sprays against *S. laxa*, which causes a brown rot disease of blossoms of stone-fruit in California.

Copper-lime-arsenic mixtures have been used as eradicant sprays against brown rot of plum in the U.S.A. Keitt and Palmiter (1937) report that four such mixtures, in small-scale exploratory tests, were highly effective in suppressing the production of conidia of *Sclerotinia fructicola* (Wint.) Rehm on overwintered mummified fruits of plum. Calavan (1944) claims a high degree of control of brown-rot blossom and spur blight of sour cherry, caused by *S. laxa*, by the use of a similar eradicant spray, particularly if supplemented by a later protectant spray of Bordeaux mixture.

Following fairly recent experiments on the control of brown rot of apricot in the Crimea, Youganova (1946) recommends the use of a spray consisting of copper naphthenate in an oil emulsion, applied several times during the season. It is also claimed that one such application effected a considerable reduction in twig damage resulting from this disease.

Protectant sprays have also been employed against brown rot of fruit, to control infection during the current season. A review is given by Marsh (1947) of recent U.S. work on new organic fungicides used as summer sprays against scab and brown rot.

In this country, Bennett *et al.* (1944) obtained an approximately four-fifths decrease in a relatively light brown rot infection of apples in Worcestershire, by the use of a Fermate (ferric dimethyldithiocarbamate) spray in late June. Subsequent experience in other localities suggests, however, that Fermate spraying will not control a heavy brown rot infection of apples (Marsh, 1947), and the persistent black deposit left by the spray is a most undesirable feature.

* Part of the work described in this report was carried out at the Long Ashton Research Station, by arrangement with the University of Reading.

## MATERIALS.

### Trees.

In the investigations described in this paper, winter spraying trials were carried out at Reading University Horticultural Station, on mummified apple fruits infected with *S. fructigena* overwintering on 40-year old bush trees of the variety Bramley's Seedling.

Tests were also carried out on detached mummified apples collected from trees of the varieties Cox's Orange Pippin, Worcester Pearmain, Newton Wonder and Bramley's Seedling. No attempt was made to segregate the varieties. Detached mummified plum fruits, infected with *S. fructigena*, of mixed varieties, were used for corresponding experiments on brown rot of plum.

A summer spraying trial was carried out at Reading University Horticultural Station on nine 40-year old standard plum trees, of the variety Belle de Louvain. These were planted at approximately 30 feet apart in two isolated rows.

### Fungicides.

*Copper naphthenate*

This material was compounded for spraying as follows :—

| | |
|---|---|
| Copper naphthenate .. .. .. .. .. .. | 6 gms. |
| Grade " G " oil .. .. .. .. .. .. | 60 gms. |
| Bentonite .. .. .. .. .. .. .. | 30 gms. |
| Water to .. .. .. .. .. .. .. | 1 litre. |

The copper naphthenate was thoroughly dissolved in the oil by gentle warming, and the resulting oily solution added, little by little, to a stiff paste of Bentonite and water, stirring vigorously to emulsify the oil before adding a further quantity. Small amounts of water were added as necessary when the emulsion became too oily. On completion, the mixture was diluted to full volume.

*Phenyl mercury chloride*

This material was used in both winter and summer sprays.

*Formula " A ".* (Winter spray)

| | |
|---|---|
| Phenyl mercury chloride .. .. .. .. .. | 3 gms. |
| Grade " G " oil .. .. .. .. .. .. | 50 ccs. |
| (emulsified with 25 ccs. of 20% Sulphite lye) | |
| Water to .. .. .. .. .. .. .. | 1 litre. |

In certain later trials, 1 cc. " Calsolene HS " wetting agent was added to the above quantity of spray after dilution (*Formula " B "*)

*Formula " C ".* (Used in one Winter spray trial)

| | |
|---|---|
| Phenyl mercury chloride .. .. .. .. .. | 3 gms. |
| 80% Miscible tar-oil emulsion .. .. .. .. | 70 ccs. |
| Water to .. .. .. .. .. .. .. | 1 litre. |

*Summer Spray*

| | |
|---|---|
| Venturicide (containing 2·5% Phenyl mercury chloride) | 2 gms. |
| Water .. .. .. .. .. .. .. | 1 litre. |

*Monocalcium arsenite*

A suspension of this material was compounded in the laboratory from arsenious oxide and calcium hydroxide by shaking together in a small quantity of distilled water for twenty minutes.

The ratios of lime and arsenious oxide were those required by the formula $CaH_4(AsO_3)_2$, and the compound will therefore be referred to as monocalcium arsenite, although, in the incomplete state of our present knowledge of the chemistry of these compounds, the possibility remains that the material may be a mixture.

Monocalcium arsenite was used at two concentrations :—

*Formula " D "*

| | |
|---|---|
| Monocalcium arsenite | 2·5 gms. |
| Grade " G " oil | 20 ccs. |
| (emulsified with 1 cc. " Perminal EML " in 10 ccs. water) | |
| Distilled water to | 1 litre. |

*Formula " E "*

| | |
|---|---|
| Monocalcium arsenite | 4 gms. |
| Grade " G " oil | 20 ccs. |
| (emulsified with 1 cc. " Perminal EML " in 10 ccs. water) | |
| Distilled water to | 1 litre. |

*Sodium arsenite*

This was obtained as such, and made up for spraying as follows :—

| | |
|---|---|
| Sodium arsenite | 1 gm. |
| Grade " G " oil | 50 ccs. |
| (emulsified with 1 cc. " Calsolene HS " in 25 ccs. water) | |
| Water to | 1 litre. |

*Copper-lime-arsenic mixture*

This was compounded as follows :—

| | |
|---|---|
| Copper sulphate crystals ($CuSO_4.5\ H_2O$) | 15 gms. |
| Calcium hydroxide | 3·3 gms. |
| Monocalcium arsenite | 7·5 gms. |
| Arachis oil (in lieu of fish oil) | 6·25 ccs. |
| (emulsified with 1 cc. " Perminal EML ") | |
| Distilled water to | 1 litre. |

The monocalcium arsenite component, prepared as before, was added last, to minimise the formation of copper arsenites.

*Lime-sulphir*

This was used for summer spraying at 1%, without the addition of a spreader.

The following materials were also tested, but proved unsuccessful in field trials :—

Tricalcium arsenite 0·25%

A copper-lime-zinc arsenite-monocalcium arsenite mixture.

Phenothiazine 0·4%

Certain chloronitrobenzene compounds, each used at 0·7%.

Certain proprietary sprays, based on the naphthenates of copper, zinc and magnesium.

The following materials were used in spore germination tests in the laboratory :—

*Phenyl mercury chloride*, mixed with forty times its weight of Bentonite.

8-*Hydroxyquinoline sulphate*, as the neutral monohydrate.

2 : 3-*Dichloro*-1 : 4-*naphthoquinone*, in the form of " Phygon XL," containing 50% of active material.

*Dinitro-ortho-cresol*, prepared by dilution of a stock emulsion containing 2% D.N.C. and 60% JD-2 petroleum oil, with sulphite lye emulsifier.

2-*Heptadecylglyoxalidine*, in the form of Experimental Fungicide 341-C, containing 54% of the glyoxalidine as an acetate salt dissolved in isoproponal. 0·25 gm. calcium hydroxide was added per cc. of Fungicide 341-C.

*Zinc dimethyldithiocarbamate*, in the form of a dispersible powder containing 80% of the pure compound.

*Zinc ethylene bisdithiocarbamate*, in the form of Dithane Z-78 dust, containing 65% of active material.

*Pentachlornitrobenzene*, in the form of a dispersible powder containing 20% of active material.

*Standard Laboratory Bordeaux Mixture*, prepared as recommended by the American Phytopathological Society, Committee on Standardization of fungicidal tests (1943b).

*Lime-sulphur*, by dilution of the commercial concentrate.

*Copper naphthenate*, prepared as for spraying.

*Ferric dimethyldithiocarbamate* as the pure compound, together with four times its weight of Bentonite.

## METHODS.

### Field Spraying—Winter (Apples).

Individual and clustered mummified apples overwintering on the trees were sprayed by means of a small atomising apparatus, as commonly used for nose and throat spraying, ensuring that the whole surface of each fruit was wetted. These trees received an otherwise normal spraying programme throughout the year, consisting of a D.N.C. wash (0·1% in 5% petroleum oil) on February 13, 1948, and three spring applications of lime-sulphur, with the exception of one group for which the winter D.N.C. spray was omitted.

All the fruits not required for earlier records were removed from the trees during early July, 1948, and the number of pustules on each counted and recorded.

### "Rack" Method (Apples and Plums).

Other mummified fruits detached from the trees in autumn were overwintered on wire-netting trays on a wooden "rack" structure approximately two feet high, constructed in the orchard. During the winter, these mummified fruits were treated by dipping into a beaker containing the appropriate fungicide, and dried on waxed wire-netting strips before being replaced on the "rack", which was protected from routine orchard spraying.

### Laboratory Method—Examination of Viability of Mycelium in Treated Fruits.

In order to study the effect of certain fungicides on the mycelium of the fungus in treated mummified apples, a small number of fruits from each treated batch was brought into the laboratory at monthly intervals during winter and early spring.

Each apple was dissected, using a sterile scalpel. One half was placed on moist filter paper in a Petri dish, and exposed to fluorescent "long-day" lighting for seventy-two hours to stimulate the formation of sporing pustules

of *S. fructigena.* If such pustules were produced, the number was recorded and the second half of the apple discarded.

If no pustules were produced from the first half, the second half, which meanwhile had been maintained at a temperature of approximately 0° C., was surface sterilised by immersion in mercuric chloride solution (1 in 2000) for three minutes, thoroughly rinsed in sterile distilled water, and then further dissected.

Three cultures from just beneath the surface, and three from the interior of the half-apple were each transferred, by means of a sterile needle, to a Petri dish containing potato extract agar, which was acidified with malic acid, at a final concentration of 0·25% in the medium, to a pH of approximately 4·4.

Each Petri dish accommodated twelve cultures, representing the surface and interior of each of two fruits, and was carefully marked and labelled accordingly. The Petri dishes were incubated at 71° F., for three days, and then transferred to "long-day" fluorescent lighting for a further three days, to encourage sporulation of the cultures and thus to aid their identification.

Records were made for each fruit of the production of sporing pustules, or, if none was produced, of the viability of the mycelium near the surface and in the interior. The fungus was considered viable if *S. fructigena* was identified in any of the three cultures.

**Field Spraying—Summer (Plums).**

The fungicides were applied on June 23rd, 1948, by means of a power sprayer (K.E.F./200/P.20, 1946 model), using a twin-nozzle broom-head. Each tree received approximately 4 gallons of spray. Conditions were favourable for spraying, and no rain fell during the ensuing seventy-two hours.

## EXPERIMENTAL.

**Main Winter Trials and Counts.**

The main winter trials were carried out with the following fungicides :—

Copper naphthenate
Phenyl mercury chloride (formula A)
Monocalcium arsenite (formula D)

Each of these sprays was applied on five dates during the winter, at intervals of approximately twenty-five days (using different mummified fruits on each occasion). Phenyl mercury chloride, plus a wetting agent (Formula B), was also included on the last three dates of application.

All available mummified apples on the trees, with the exception of a small number left unsprayed as Controls, were thus treated, together with corresponding batches of mummified apples on the "rack."

Pustules were first observed on the fruits on the trees on May 27th, 1948, and, on July 7th, pustule production from all remaining fruits was recorded, with the results shown in Table I. It was noticeable during these recordings that the majority of pustules were on the upper parts of the fruits.

TABLE I.

PRODUCTION OF PUSTULES BY MUMMIFIED APPLES ON TREES.

| (1) Fungicide | (2) Date of application | (3) Record on July 7 of no. of fruits examined bearing pustules | (4) Mean no. pustules per fruit examined |
|---|---|---|---|
| Copper naphthenate 0·6% | Dec. 8 | 3/3 | 101·7 |
| | Jan. 14 | 4/5 | 62·0 |
| | Feb. 9 | 3/5 | 27·8 |
| | Mar. 1. | 2/3 | 28·0 |
| | Mar. 23 | 3/4 | 28·5 |
| | TOTAL (all dates) | 15/20 | Mean 47·6 |
| Phenyl mercury chloride Formula "A" | Dec. 19 | 1/5 | 1·0 |
| | Jan. 14 | 1/5 | 7·6 |
| | Feb. 9 | 2/5 | 7·4 |
| | Mar. 1 | 1/3 | 2·3 |
| | Mar. 23 | 0/4 | 0· |
| | TOTAL (all dates) | 5/22 | Mean 3·95 |
| Phenyl mercury chloride Formula "B" | Feb. 9 | 1/5 | 0·4 |
| | Mar. 1 | 0/4 | 0· |
| | Mar. 23 | 0/2 | 0· |
| | TOTAL (all dates) | 1/11 | Mean 0·18 |
| Monocalcium arsenite Formula "D" | Dec. 8 | 0/3 | 0· |
| | Jan. 15 | 6/7 | 10·9 |
| | Feb. 9 | 0/3 | 0· |
| | Mar. 2 | 0/3 | 0· |
| | Mar. 23 | 0/4 | 0· |
| | TOTAL (all dates) | 6/20 | Mean 3·80 |
| CONTROL | — | 6/6 | Mean 71·17 |

*Note :—*

1. No measurable rainfall was recorded on the above dates, with the exception of Jan. 14th (0.02″) and Jan. 15th (0.04″).

2. The left-hand figure in each entry in column (3) denotes the number of fruit bearing pustules, while the right-hand figure represents the total number of fruit examined.

There appeared to be a considerably smaller pustule production on trees which received the routine orchard D.N.C. spray, than on those which did not. Summarised figures for each eradicant fungicide, with and without D.N.C., are given in Table 2.

TABLE II.

EFFECT OF ROUTINE D.N.C. WASH ON PUSTULE PRODUCTION BY MUMMIFIED APPLES TREATED WITH ERADICANT FUNGICIDES.

| Fungicide | Fraction of fruits examined bearing pustules | | Mean no. of pustules per fruit examined | |
|---|---|---|---|---|
| | Sprayed DNC | Not sprayed DNC | Sprayed DNC | Not sprayed DNC |
| Copper naphthenate | 8/12 | 7/8 | 24·3 | 82·5 |
| Phenyl mercury chloride Formula "A" | 1/13 | 4/9 | 1·15 | 8·0 |
| Phenyl mercury chloride Formula "B" | 1/7 | 0/4 | 0·3 | 0· |
| Monocalcium arsenite Formula "D" | 1/7 | 5/13 | 2·0 | 4·8 |
| Control | 5/5 | 1/1 | 53·4 | 160·0 |

**"Rack" Trial.**

Pustules were first observed in large numbers on "rack" apples on May 12th, 1948, following 1·33″ of rain and a maximum temperature of 70° F. on May 11th. Pustule production, recorded on July 6th, is shown in Table 3. Each batch consisted of twelve fruits.

TABLE III.

PRODUCTION OF PUSTULES BY MUMMIFIED APPLES ON "RACK" (MAIN TRIALS).

| Fungicide | Date of application | No. of fruits bearing pustules out of twelve examined on July 6th | Mean no. of pustules per fruit examined |
|---|---|---|---|
| Copper naphthenate 0·6% | Dec. 8 | 10 | 61·7 |
| | Jan. 8 | 8 | 47·0 |
| | Feb. 4 | 9 | 43·7 |
| | Mar. 2 | 10 | 55·7 |
| | Mar. 23 | 10 | 29·6 |
| | TOTAL (all dates) | 47/60 | Mean 47·5 |
| Phenyl mercury chloride Formula "A" | Dec. 19 | 0 | 0 |
| | Jan. 8 | 1 | 0·2 |
| | Feb. 4 | 8 | 14·7 |
| | Mar. 2 | 4 | 8·2 |
| | Mar. 23 | 0 | 0 |
| | TOTAL (all dates) | 13/60 | Mean 4·63 |
| Phenyl mercury chloride Formula "B" | Feb. 4 | 6 | 9·7 |
| | Mar. 2 | 1 | 1·3 |
| | Mar. 23 | 0 | 0 |
| | TOTAL (all dates) | 7/36 | Mean 3·67 |
| Monocalcium arsenite Formula "D" | Dec. 8 | 2 | 0·7 |
| | Jan. 8 | 5 | 4·9 |
| | Feb. 4 | 1 | 8·6 |
| | Mar. 2 | 2 | 4·5 |
| | Mar. 23 | 6 | 2·3 |
| | TOTAL (all dates) | 16/60 | Mean 4·20 |
| CONTROL | — | 12/12 | Mean 59·50 |

*Note :—*

1. No measurable rainfall was recorded for the above dates of application.
2. It will be seen that the results of Table 3 follow the general trend of those of Table 1, for "Tree" fruits; the latter results, however, must naturally be considered the more valuable in any comparison of fungicidal efficiency.

**Subsidiary Winter Trials and Counts.**

Certain other fungicides were applied to batches of fourteen mummified apples on the rack on February 11th, 1948. The rainfall recorded for that day was 0·01". Pustule production was recorded on July 6th, with the results shown in Table 4.

TABLE IV.

PRODUCTION OF PUSTULES BY MUMMIFIED APPLES ON RACK (SUBSIDIARY TRIAL).

| Fungicide | No. of fruits out of 14 showing pustules | Mean no. of pustules per fruit examined |
|---|---|---|
| Monocalcium arsenite 0·4% Formula "E" | 1 | 0·07 |
| Sodium arsenite 0·1% | 0 | 0 |
| Copper-lime-arsenic mixture | 1 | 0·36 |
| Control | 9 | 49·71 |

A similar experiment was carried out on batches of detached mummified plums on February 13th, 1948, the plums in this experiment being dried off in the laboratory before removal to the rack five days later. Details of pustule production, recorded on July 5th, are shown in Table 5. The figures include pustules of *S. laxa* observed on a few fruits.

TABLE V.

PRODUCTION OF PUSTULES BY MUMMIFIED PLUMS ON RACK (FIRST TRIAL).

| Fungicide | No. of fruits out of 16 showing pustules | Mean no. of pustules per fruit examined |
|---|---|---|
| Copper naphthenate 0·6% | 7 | 5·8 |
| Phenyl mercury chloride Formula "A" | 3 | 0·5 |
| Phenyl mercury chloride Formula "B" | 0 | 0 |
| Monocalcium arsenite Formula "D" | 0 | 0 |
| Copper-lime-arsenic mixture | 0 | 0 |
| 80% Miscible tar-oil emulsion at 7% | 5* | 12·0 |
| Control | 12 | 21·6 |

* 8 fruits only examined.

Further mummified plums later became available, and batches of sixteen were treated on March 10th, being dried in the laboratory for 24 hours before transfer to the "rack." Results of pustule production, recorded on July 5th, are shown in Table 6: it will be seen that the general level of viability of the fungus in this experiment was low, as shown by the results for Control.

TABLE VI

PRODUCTION OF PUSTULES BY MUMMIFIED PLUMS ON RACK (SECOND TRIAL).

| Fungicide | No. of fruits out of 16 showing pustules | Mean no. of pustules per fruit examined |
|---|---|---|
| Phenyl mercury chloride Formula "B" | 0 | 0 |
| Phenyl mercury chloride Formula "C" | 0 | 0 |
| Control | 9 | 8·4 |

**Laboratory Trial.**

Examination of treated mummified fruits from both trees and rack were commenced on the following dates (1948): January 12th, January 30th, February 20th, March 22nd and April 20th.

Table 7 shows that there was a marked difference between the results of the first three examinations and those of the last two, in respect of the viability of the mycelium of *S. fructigena* in treated fruits not producing pustules; the proportion of fruits containing no viable mycelium was much greater in the last two examinations. It will be seen, however, that the percentage of fruit which produced pustules remained almost constant for each treatment.

A possible explanation of this feature is provided by the spell of exceptionally dry weather in late winter; from February 15th to March 14th (1948)

inclusive, the rainfall recorded at Reading University Horticultural Station was only 0·08″, of which melted snow accounted for 0·06″, and easterly winds, generally of relatively low humidity, prevailed on twenty out of the twenty-eight days. The resulting desiccation of the mummified fruits, which was apparent from their decrease in size, may have caused the complete death of the mycelium in fruit where it had already been largely eradicated by fungicidal treatment.

A notable feature of fruits treated with phenyl mercury chloride was the large proportion whose surface was sterile, as shown by the culturing technique. Fruits treated with monocalcium arsenite, however, showed a high degree of contamination with *Penicillium spp.* Although eradicating *S. fructigena*, the fungicide presumably did not kill the *Penicillium spp.*, and may even have had a stimulatory effect.

TABLE VII.

Summary of Results of Laboratory Examinations of Mummified Fruits Treated with Eradicant Fungicides.

| Fungicide | Examinations summarised | Total No. fruits examined | Fruit showing pustules | | Fruit not showing pustules | | |
|---|---|---|---|---|---|---|---|
| | | | No. | % of Total | No. with mycelium viable surface & interior | No. with mycelium viable interior only | No. with mycelium non-viable |
| Copper naphthenate 0·6% | First, second & third. | 21 | 13 | 62 | 6 | 1 | 1 |
| | Fourth & fifth | 34 | 21 | 62 | – | – | 13 |
| Phenyl mercury chloride Formula "A" | First, second & third. | 20 | 6 | 30 | 1 | 8 | 5 |
| | Fourth & fifth | 25 | 7 | 28 | – | 2 | 16 |
| Phenyl mercury chloride Formula "B" | Third | 3 | – | – | – | 1 | 2 |
| | Fourth & fifth | 16 | 2 | 12 | – | 2 | 12 |
| Monocalcium arsenite Formula "D" | First, second & third | 21 | 2 | 10 | 4 | 3 | 12 |
| | Fourth & fifth | 37 | – | – | – | 1 | 36 |
| Control | First, second & third | 11 | 5 | 45 | – | 1 | 5 |
| | Fourth & fifth | 6 | 3 | 50 | – | – | 3 |

*Note* :—

In no fruit was mycelium of *S. fructigena* found viable at the surface, yet non-viable in the interior.

**Summer Trials.**

As previously explained, this trial was designed to explore the immediate effects of summer spraying. On August 12th, 1948, 200 or 250 fruits were picked from each of the nine plum trees sprayed on June 23rd, taking them from a convenient cone-shaped section of each tree, extending to the centre. Fruit showing any brown rot infection was classified as infected. Table 8 shows the results of the count.

TABLE VIII.

Brown Rot Infection of Belle de Louvain Plum on August 12th, 1948.

| Treatment | Tree code No. | No. fruit picked | No. infected |
|---|---|---|---|
| Lime-sulphur 1% | S.1 | 250 | 32 |
| | S.2 | 250 | 15 |
| | S.3 | 250 | 23 |
| | Mean percentage infection = 9·7% | | |
| Venturicide 0·2% | V.1 | 250 | 30 |
| | V.2 | 250 | 9 |
| | V.3 | 250 | 3 |
| | Mean percentage infection = 5·6% | | |
| Control | Z.1 | 250 | 74 |
| | Z.2 | 200 | 32 |
| | Z.3 | 250 | 51 |
| | Mean percentage infection = 22·0% | | |

Chi-square analysis (Brandt and Snedecor) of the data of Table 8 suggests that, while there is considerable heterogeneity within treatments, there is also a significant difference between treatments as a whole. Further comparison between pairs of treatment suggests that Venturicide is a significantly better treatment than Lime-Sulphur, and that the latter treatment is significantly better than Control. The reductions in percentage of mean infection in these samples resulting from the Venturicide and Lime-Sulphur treatments were approximately three-quarters and one-half respectively.

Lime-Sulphur, but not Venturicide, left a white deposit conspicuous at picking time.

**Phytotoxic Effects.**

*Winter Sprays*

In order to obtain preliminary information on the phytotoxicity of the sprays tested in the winter trials, each fungicide was also sprayed on a number of healthy twigs on trees of Bramley's Seedling apple and miscellaneous varieties of plum and pear at the same time as it was used on mummified fruit on trees and rack ; any subsequent damage was recorded. No such damage resulted from any spray applied up to and including March 2nd, 1948.

The March 23rd application of copper naphthenate and monocalcium arsenite sprays caused damage to apple buds, then in the " Burst " stage, and severe damage to plum and pear buds (then in the " White Bud " and " Burst " stages respectively). Both plums and pears were also injured to a lesser extent by the phenyl mercury chloride sprays (Formulae A and B) of that date.

*Summer Sprays*

No damage was recorded from the 1% Lime-sulphur and 0·2% Venturicide sprays applied to plum trees on June 25th, 1948.

**Preliminary Spore Germination Tests.**

The test-tube dilution slide-germination method, as recommended by the American Phytopathological Society, Committee on standardization of fungicidal tests (1943a, 1947) was used for these experiments, with details as follows :—

*Fungus.* Spores were taken from 7-day old cultures, grown at 25° C., of *S. laxa* on autoclaved potato plugs previously soaked in 7½% malic acid for 72 hours.

*Spore Stimulant.* 0·1% orange juice. In certain later tests, it was found necessary to use 0·2 millimolar $MgSO_4$ in addition.

*Germination Period.* 17 hours at 25° C.

LD-50 values, based on one test only with each fungicide, are shown in Table 9. Further tests are in progress.

TABLE IX.

LD-50 Values of Certain Fungicides against *S.Laxa.*

| Fungicide | Fungistatic LD-50 value (p.p.m.) |
|---|---|
| Phenyl mercury chloride (with Bentonite) .. .. .. | 0·17 |
| 8-Hydroxyquinoline sulphate .. .. .. .. | 0·37 |
| 'Phygon XL' (containing 50% 2:3-dichloro-1:4-naphthoquinone) .. | 0·39 |
| Standard Laboratory Bordeaux Mixture (expressed as Cu) .. .. | 0·74* |
| Experimental Fungicide 341-C (containing 54% glyoxalidine) plus lime | 0·88* |
| Ferric dimethyldithiocarbamate (with Bentonite) .. .. .. | 9.8 |
| Dinitro-ortho-cresol (in oil emulsion) .. .. .. .. | 11·0 |
| Zinc dimethyldithiocarbamate (80% preparation) .. .. .. | 11·0* |
| Lime-sulphur .. .. .. .. .. .. .. | 15·0 |
| Dithane Z-78 (containing 65% zinc ethylene bisdithiocarbamate) .. | >100·0 |
| Pentachlornitrobenzene (20% active material) .. .. .. | >100·0 |
| Copper naphthenate (in oil emulsion) .. .. .. .. | >100·0 |

* With 0·2 millimolar $MgSO_4$ as spore stimulant.

## DISCUSSION.

The results of the preliminary spore germination tests have shown the high fungistatic properties to *S. laxa* of phenyl mercury chloride, 8-hydroxyquinoline sulphate, 2:3-dichloro-1:4-naphthoquinone and 2-heptadecylglyoxalidine. Of these compounds, only phenyl mercury chloride was used in field experiments. Copper naphthenate and the arsenicals were included in the winter trials because of the claims made for them by other workers.

An outstanding feature of the results of the field trials has been the consistent failure of copper naphthenate winter sprays to achieve satisfactory inhibition of pustule production.

Phenyl mercury chloride has shown considerable promise during these trials, both as a winter and as a summer spray. As a winter spray it has the great advantage over the arsenites that it is comparatively safe and pleasant to use, and, while there is some evidence to suggest that it is also less phytotoxic, its toxicity to *S. fructigena* in laboratory tests is outstanding.

Early laboratory examinations of treated fruit, however, showed that it was erratic in its action on the mycelium of *S. fructigena* in mummified apples. The surface of some of these appeared to be completely sterile, while vigorous pustules were produced from parts of the surface of others. The possibility that inperfect wetting might be the cause led to the incorporation of a wetting agent

in the spray, with improved results : those obtained with the modified spray (Formula B) for fruits on the trees, as shown in Table 1, are particularly promising, although the number of fruit involved was small.

As a summer spray, phenyl mercury chloride (as Venturicide) gave a good degree of control of the current season's brown rot of plum in a small scale trial : the result is especially encouraging in view of the fairly high level of infection, as shown by the figure for Control (22·0%), while the absence of visible spray residue is a further advantage.

Monocalcium arsenite at 0·25% (Formula D) showed good eradicant properties ; the results for fruits on the trees, as shown in Table 1, indicate that only when appreciable rain followed the application of the spray (as on January 15th, 1948) were any pustules produced by treated fruits. Sodium arsenite and the copper-lime-arsenic mixture were both effective in subsidiary small-scale trials (see Table 4).

The killing of the mycelium throughout the mummified tissue of the fruit requires penetrative properties in the fungicide, and there is some indication that monocalcium arsenite is of particular value in this respect, although further experiments are required to confirm this.

In this country, it would be of considerable value, from the point of view of the grower, if it were possible to control the brown rot diseases by means of a small modification of the existing spray programme, such as the addition of some material to present winter washes. The use of the arsenites for this purpose is open to serious objections, owing to their poisonous nature, but the results of the experiments described suggest that phenyl mercury chloride may fulfil the necessary requirements, and more extensive experiments are being carried out on these lines.

In conclusion, it is emphasised that the results of the winter spraying trials described refer only to the inhibition of spore formation by mummified fruits, and not to the resulting control of fruit infection, which, in general, will be considerably less effective, owing to the ability of the disease to spread rapidly from a small source of infection, under favourable conditions (Wormald, 1935 ; p. 42).

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U.S. Rubber Co.—2:3-Dichloro-1:4-naphthoquinone.

Carbide and Carbon Chemicals Corporation (N.Y.)—2-Heptadecylglyoxalidine.

Imperial Chemical Industries Ltd.—Zinc dimethyldithiocarbamate.

Rohm and Haas Co.—Zinc ethylene bisdithiocarbamate.

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# SOME RECENT STUDIES ON THE NATURE AND INCIDENCE OF CIDER SICKNESS.

By B. T. P. BARKER.

Since investigations on the bacterial disorder of Cider Sickness were started at this Station over forty years ago and the first detailed account of the work published in 1912, (1), further studies on various aspects of the subject have been recorded from time to time in the Annual Reports of the Station and elsewhere, (2), (3), (4), (5), (6), the last appearing in 1925. (7) Shortly after that date the stock culture of the causal organism used in the experimental work throughout that period and first isolated in 1911 died. This caused a cessation of further investigations until 1943, when a severe and widespread outbreak of sickness occurred among the ciders made at the Station in the autumn of 1942. The causal organism in these cases was isolated in the spring of 1943. Further isolations from sick ciders made at the Station in 1947 followed in 1948. Important new light upon the disorder has appeared since work upon it was restarted in 1943 : although these investigations are still unfinished, it seems desirable to report the results so far achieved. The significance of these results in relation to the nature of the disorder is such that some preliminary reference to its character and incidence, and to current methods for its prevention and control in practice, is necessary.

The original investigations were started at a time when cider-making in England was still largely carried on by the West Country farmers. Apart from the quantity reserved for consumption on the farm the cider was sold in most cases locally as a farm product surplus, but frequently over a wider field by farmers producing it as a regular and profitable commercial commodity. Several of the latter specialised to such an extent that their farming became a secondary matter. Their ciders were characteristic of the districts in which they were located, the fruit supplied being generally obtained from the immediate neighbourhood. With the introduction of efficient filters a high standard of quality was reached in those areas where, on account of the soil character and the nature of the local varieties of apples, juices of high specific gravity and low acidity and rate of fermentation predominated. Such juices, while yielding the richest ciders, were for the same reason most susceptible to the sickness disorder. Thus, at that period in the evolution of the industry, sickness became its greatest menace and a cause of serious loss to the individual makers concerned. This disorder at that time was commonly called " Second Fermentation."

The result of this initial research showed that the most effective suitable methods of preventing outbreaks of the disorder were (a) to raise the acidity level of the juices or ciders to a point not less than 0·5 per cent (expressed as malic acid) by blending with high-acid juices or ciders or by the addition of tartaric or citric acids, and (b) to maintain some degree of yeast activity in the liquor until its final transfer to bottle. By those means the number of outbreaks was reduced considerably, although they were not entirely eliminated.

Since those days the character of the industry has undergone a great change. Now cider-making on the farm has dwindled to a relatively small output from the commercial point of view, although some of the keener makers of that class have expanded their business into factory concerns of substantial

size. The main sources of supply, however, are the larger *ad hoc* factories with outputs of many thousands of gallons annually. Production on that scale necessitates the drawing of cider fruit supplies from wide areas and also supplementary supplies of cull eating apples—normally yielding high-acid and rapidly fermenting juices—from commercial fruit growers of the Eastern and South-Eastern counties. Under such conditions the chances of sickness outbreaks have been very greatly reduced. Nevertheless a real risk remains for producers of ciders retaining some degree of natural sweetness, especially for those aiming at the production of the rich sweet full-bodied and fruity beverage which approaches the peak of cider vintage quality.

Thus it is important that research on the subject should proceed until the major outstanding problems are solved, especially since many consumers find ciders with the protectant acidity standard too sharp for the palate.

It is not less vital also for the future of cider research at Long Ashton, since that inevitably will require the use of single-variety juices, many of which are of the sickness-susceptible type. Hence even a trace of sickness, so slight as to be unnoticed, not only might ruin an experiment but also be a cause of risk of mis-interpretation of results.

## THE NATURE OF CIDER SICKNESS.

Summarising briefly the outstanding characters of the disorder as revealed by the earlier investigations, the attacked ciders—all of which have retained a sufficient sugar content to permit active growth of the causal organism—undergo a sudden and violent fermentation after the normal primary yeast fermentation has ceased naturally, or has been arrested by the removal of the yeast crop. Copious production of carbon dioxide and formation of ethyl alcohol to the extent of about 50 per cent of the sugar destroyed occur. Accompanying features are very characteristic changes in the flavour and aroma of the cider, due largely to the formation of acetaldehyde and other aldehydes, these being followed more or less rapidly by the development of a dense turbidity—probably resulting from the action of the aldehydes on the tannins of the ciders to form an insoluble derivative or derivatives.

Several variants of these features have been noted in individual cases and have also been obtained with pure cultures of the causal organism in suitable artificial culture media. For example, the aroma and flavour may appear without any material accompanying production of carbon dioxide or material loss of sugar : at other times the sugar has been destroyed with equivalent alcohol production but complete absence of carbon dioxide formation. In occasional instances the characteristic aroma and flavour have been observed even in the apples themselves when apparently sound, but somewhat over-ripe.

Normally the disorder appears in ciders made from juices of low organic nitrogen content, which in consequence show a very slow rate of primary fermentation : frequently they cease fermentation completely quite early in life, leaving a substantial quantity of residual sugar unfermented. Although occasionally ciders with an acidity equivalent to more than 0·5 per cent acid are attacked, outbreaks are almost invariably confined to those of lower acidity—especially in the region of about 0·25 per cent, the usual approximate figure for ciders made from sweet and bittersweet apples exclusively.

The summarised description of the disorder just given is based upon details recorded in the papers mentioned above and the chemical data relate to the action of the causal organism originally isolated.

## THE CAUSAL ORGANISMS OF SICKNESS.

The occasional variations in the features of the disorder have raised doubts from time to time as to that organism being the sole causal agent. Since, however, analagous variations had occurred when the organism had been used in pure culture, there had seemed hitherto no justification for assuming the existence of other sickness bacteria. The organisms isolated from sick ciders in 1943 and 1948 provided an opportunity of testing the point.

In the case of the 1943 outbreak a set of malt-extract gelatine fractional plate cultures was prepared after infection with a drop of the affected cider. From the last and thinnest sown plates of the series stock cultures were prepared from ten separate bacterial colonies. These were kept under observation for a period of two years, during which period examination of their distinctive characters was made. In each instance the responses to the individual tests were the same. It was concluded, therefore, that the cultures were replicates of a single organism. The tests included inoculations of ciders of various types and the usual symptoms of the sickness disorder appeared in all the ciders of the susceptible class after infection.

Five years later further isolations were made from another cider made at the Research Station the previous autumn which had succumbed to an outbreak of sickness in the spring following. In this instance single-colony isolations were made independently by Dr. Challinor and the author. Subsequent comparisons showed the two isolates concerned to be identical. This organism also proved to be able to induce sickness in ciders of the low-acid class.

Collateral tests with the 1943 and 1948 isolates were then carried out. It was found that in day-old cultures in nutrient solutions such as malt extract and glucose-yeast extract at 25° C. the individual cells of the two organisms were indistinguisable in appearance. In size about 2 $\mu$ in length and 1 $\mu$ in diameter, they occur both singly and paired as diploids. Frequently in very vigorous cultures the cells are more elongated. Involution forms are found in old cultures.

In spite of the similarity in appearance of the cells in single and paired arrangement, there is one feature in cell arrangement which makes it easy to distinguish between the two organisms by microscopic examination of both young and old cultures. The 1943 strain so far has shown no variation from the cell forms just mentioned except that chains of two or more pairs attached end-to-end can be found in fair numbers in liquid cultures a day old or more. Similar compound chains of cells occur with the 1948 strain, but in its case more complex cell aggregations constitute a characteristic feature. Instead of the cell unions lying in one dimension only, the adherent cells in these aggregates are attached in such a way as to form ball-like masses or irregular clumps of cells. Such clumps are numerous in liquid cultures a day old or more. This character has remained a constant feature since the original isolation of the bacterium in the spring of 1948 and it seems permissible, therefore, to regard it as a specific feature of the strain.

In both strains the extreme motility of the cells in young liquid cultures is a noteworthy feature. This can be seen in the most striking manner under the microscope in individual colonies of cells grown in a hanging-drop culture of a suitable gelatine nutrient medium, such as glucose-yeast extract with a gelatine strength of 10 per cent. After two or three days growth at ordinary laboratory temperature some of the more superficial colonies of the drop show a sufficient degree of liquification of the immediate zone of gelatine

encircling the individual colonies to permit motility of the cells. The picture is one of intense activity, recalling the most active swarming of a hive of bees. In addition to the high-speed straight-ahead swimming of individual cells across the field of vision of the microscope in liquid cultures a much slower, sinuous type of movement is exhibited by some of the more elongated diploids. Another characteristic form of movement is shown by some single cells, these spinning round at a great pace on their own axis without any material forward motion.

Vigorous motility in liquid cultures is not of long duration and appears to be associated with the peak point of active cell multiplication. Both these features are found most highly developed in nutrient solutions containing one or both of the two carbohydrates fermented freely by these organisms, viz., glucose and fructose.

In a test-tube culture containing 10 mls. of a 5 per cent solution of either of these sugars and 1 per cent yeast extract active growth and fermentation are apparent within 24 hours at 25° C. Within the next 48 hours the fermentation ceases and the turbidity of the liquor—due to the actively motile cells of the organism—begins to decrease as the cells become immobile and sink to the base of the tube. Immobile cells lose their viability quickly. In week-old liquid cultures at 25° C. a few motile cells still remain : their numbers continue to diminish as the culture ages and within two or three weeks only rare ones can be found. When motility has completely disappeared from a culture, transfer of the organism to fresh fluid culture media is rarely successful in reviving it. Thus, unless stock cultures are renewed at frequent intervals, the risk of losing the organism is considerable. The most satisfactory method so far examined of maintaining a viable stock culture over a fair period is by making a shake culture in a test-tube of a 10 per cent gelatine culture medium, such as malt extract : the submerged colonies, which develop very little in size, can be relied upon to retain their viability for at least three months. In such cultures, when a glucose-containing medium is used, the gel is disrupted to some extent by the pressure of the carbon dioxide produced within the medium by the fermentation of the sugar by the submerged colonies : frequently the disruption is so violent that the upper portion of the gelatine in the tube is blown as far as the cotton wool plug sealing the tube, and in some cases the plug has been forced out of the tube.

Until the investigation of the microbiological and chemical characters has been completed a full comparison of the two organisms cannot be made ; but an examination of the two forms in relation to some of the members of the Vitamin B complex has disclosed that there is at least one point of difference of a bio-chemical nature between them. This will be dealt with in a later paper.

Unfortunately owing to the loss of the stock culture of the original sickness bacterium referred to above it was not possible to compare this with the 1943 and 1948 strains by collateral tests. The description of the original form reveals unquestionably that the respective isolates are not identical in all their characters. One outstanding difference is so marked that it alone suffices to establish that point ; it is as follows. When grown in nutrient solutions containing glucose as the carbohydrate, the original organism produced an aroma so characteristic that it could not fail to be recognised and so intense that it could be detected without difficulty through the thick cotton wool plug of the culture vessel. The other two organisms on the other hand, when grown in media of the same kind, produce very little aroma and that lacks entirely any resemblance to that of the former case.

Thus from three sick ciders a different organism has been isolated from

each. The three bacteria concerned are closely alike in size, appearance and shape. Each is actively and characteristically motile in young liquid cultures and quickly dies after cessation of motility. No spore formation has been noted in either case. The outstanding features of the fermentation produced by them in cider are practically identical and the classes of cider most susceptible to attack are the same in each instance. The chemistry of the fermentation, whether in cider or synthetic liquid culture media, is distinctive and, as so far examined, of the same general character for all three forms. The difficulty of preservation of viability in stock cultures is another feature in common.

With such a series of close resemblances in their characters they must be classified as belonging to the same genus. The distinguishing differences, too, appear to be of a relatively minor order and it is questionable if they are sufficient to justify the separation of the three forms into distinct species. Provisionally, until their microbiological characters have been studied more closely, the most appropriate course seems to be to regard them as distinct varieties or strains of a definite species and to refer to them as Strains A (1911 isolate), B (1943 isolate), and C (1948 isolate) respectively.

But, however they may be treated taxonomically, it is clear that sickness can no longer be regarded as a specific disorder of cider resulting from the action of one individual kind of organism. In future the designation " sickness " must be considered as a general one, applying to a type of fermentation distinguished by the breakdown of certain sugars to yield not only ethanol but also aldehydes—especially acetaldehyde—in sufficient quantity to justify its appellation as " aldehydic " fermentation. In fact, the case seems closely parallel to that of acetic fermentation : as in that instance, the number of organisms capable of producing it is indefinite.

In the light of this conclusion it is to be expected that some variation in the precise character of individual outbreaks of sickness and the conditions associated therewith should occur from time to time ; it is interesting to note that past experience of the disorder has shown that variability and uncertainty are two outstanding features.

## CONDITIONS AFFECTING OUTBREAKS.

Possibly the greatest uncertainty in relation to sickness in practical cider-making hitherto has been the apparently haphazard distribution of outbreaks among individual ciders which, from their composition and general character, have been considered open to attack. This class includes typically ciders of low acidity—below 0·45 per cent malic acid—and pH ranging from about 3·8 to 4·5, made from juices with low initial organic nitrogen content (less than about 0·007 per cent), and with residual unfermented natural sugar of the order of 4 per cent upwards. The usual experience is that, while some develop the disorder actively, the remainder remain more or less resistant or entirely immune. The absence of an outbreak in an individual case does not imply the absence of the causal organism, for the chances of escape from infection at one stage or another of the making process are remote. It is indeed possible that all freshly-expressed juices are infected direct from the fruit.

Two possible explanations for the resistance to an outbreak of sickness shown by certain ciders of the low-acid class immediately suggest themselves. One is that resistance is due to the presence of a toxic factor of some kind : the other, that the conditions for the nutrition of the sickness organism are inadequate for the necessary degree of its growth and multiplication.

The former view may appear to receive support from the well-known fact that the maintenance of yeast activity in at least some slight degree is

one of the most effective methods for prevention of outbreaks. The precise nature of its deterrent effect is not known, but the production by the yeast of a toxin harmful to the sickness bacterium is at least a possibility to be reckoned with unless and until disproved. On the other hand, all ciders, including those which succumb to sickness, have undergone some degree of yeast fermentation, a fact which does not seem to be in accord with the "toxin" hypothesis.

**Microbiological Assay Investigations.**

For the purpose of investigation of the factors concerned with resistance against sickness, an examination of the "defective nutrition" aspect seemed the more likely to yield positive results. The most recent work in that direction at Long Ashton was suggested by the results obtained at the Station by Mrs. James (8) a year ago on the Vitamin B content of certain ciders. These showed an absence of pantothenic acid in some and riboflavin in others, while biotin and nicotinic acid were found in all. A trial with the sickness organisms B and C was made, therefore, by her to ascertain their requirements in respect of these four members of the Vitamin B complex. Microbiological assay methods were used, the culture fluids being those specified for each individual vitamin by Barton-Wright (9) and the test organisms the sickness bacteria B and C in place of the usual standard lactic acid bacteria. The first trials showed a complete absence of growth of the test organisms.

The subsequent bio-assay investigations have been continued by Miss Crang in collaboration with the writer, owing to the retirement of Mrs. James from her post at this Station.

A second series of trials on similar lines was made, but in this case the pH of the media was adjusted to 3·8, since a simultaneous test with an unfermented apple juice (Woodbine) of that pH was included. Again the microbiological assay culture fluids yielded no response in the form of active growth of the test organisms. On the other hand, the Woodbine apple juice series showed free growth combined with vigorous motility and evolution of gas. The vigour of these reactions was comparable with that of the corresponding features in the control nutrient solution (glucose-yeast extract) normally used in this work to provide supplies of the sickness organisms for inoculation purposes.

These two failures with the bio-assay culture solutions have led to a closer examination of those fluids in relation to the organisms in question, since from their composition it had been expected that some growth at least would have been obtained. This work is still in progress and will be reported upon in a later paper when more complete : but it has already shown that the presence of molybdenum as a trace element appears to stimulate free growth and active motility of the organisms in the culture solutions.

Very little, if anything, appears to be known about the presence or amount of molybdenum in apple juices and cider ; so an investigation on this subject in relation to the degree of susceptibility of individual ciders to sickness has been started in conjunction with Drs. E. J. Hewitt and D. J. D. Nicholas.

**Effects of Heat Treatments of Ciders.**

In the course of a series of direct inoculation tests with different ciders covering a wide range of specific gravities, acid and tannin content, and rate of fermentation, it was noted that in no case did sickness develop after infection when the ciders were previously heated to 100° C. Yet all of them, with one exception, have succumbed to the disorder at one time or another during the course of a series of various other forms of treatment : the one

exception was a Morgan Sweet cider affected with the disorder of oiliness.

Following this result, an examination of the influence of heating the ciders at other temperatures prior to inoculation is now in progress. The earliest results have shown that heating at 45° C. for five minutes has little or no effect, but from 50° upwards the cider becomes definitely resistant to the disorder.

In this connection it may be recorded that Dr. A. H. Campbell when carrying out some work on Strain B in 1943 in the Botanical Department of Bristol University reported to the author that he had been unable to obtain growth of the organism in sterilised ciders.

Possibly the heating effect may be associated with the destruction of enzymes in the ciders, but the latest results to hand suggest that the action of temperature is of a more complex order than a simple disrupture of the enzyme system. For instance, while all the ciders thus treated failed to show any signs of fermentation or active growth of the organism after being treated prior to infection for five minutes in the temperature range 55° C.—75° C., in one case—Kingston Black—after treatment at 100° C. slight growth and fermentation was observed three weeks later. Concurrent treatment at 90° C. gave a similar result after a further interval of four days, and after another week the cider heated to 80° C. followed suit. It may be significant that the titratable acidity of this cider was higher than that of any of the other ciders in the series except Stoke Red, which was too acid for the growth of the organism without complete or partial neutralisation of the acid.

The effect of neutralisation of the acid by addition of calcium carbonate, before and after heating to 100° C. for five minutes, was then tested on six ciders—three sharp varieties (Stoke Red, Bramley's Seedling and Gatcombe) and three low-acid bittersweet varieties (Yarlington Mill, Belle Norman and Ashton Brown Jersey). The results in this trial showed that active fermentation occurred in all cases where the acidity was neutralised, whether before or after heating. In the control set, where the juices were heated to 100° C. but the acid not neutralised, the three low-acid bittersweet varieties fermented more or less actively, but fermentation was slower in making its appearance than in the neutralised cases; the three sharp varieties had shown no signs of fermentation by ten days after inoculation. The other striking feature was that neutralisation of the acidity with calcium carbonate before heating the ciders resulted in all cases in quicker onset of fermentation than in the series where neutralisation occurred after the heat treatment: the vigour of the fermentation appeared to be somewhat greater in the former cases, but definite tests on this point have not been made yet.

Further work is necessary to ascertain the nature of the changes brought about in the ciders by the heat treatments here reported upon, but this line of investigation has already brought to light the important practical point that ciders heated to the usual pasteurisation temperature of 140° F. (60° C.) are apparently rendered immune to the disorder.

## CONCLUSIONS AND SUMMARY.

The current investigations have shown already several new facts with regard to the "sickness" disorder. It is evident that, owing to the greatly increased use of the highly acid commercial cull apples during recent years resulting from the diminishing supplies of cider fruit of low acidity, the incidence of the disorder has been reduced considerably. On the other hand, as the proportion of low-acid cider apples rises again in due course when the fresh plantings of those varieties now in progress come into bearing, there will be a

corresponding tendency for the disorder to become more prevalent unless the necessary precautions to render the product resistant to it are taken.

It has been shown that the earlier view that the disorder is the result of the action of a single specific organism needs modification. At least three different bacteria capable of producing the characteristic symptoms have now been isolated. While these differ in minor details in their behaviour, the general nature of their action is clearly alike in each instance. It appears likely that, as in the comparable disorder of acetification, the sickness features—especially in relation to the production of aldehydes—will be found to be associated with a still wider range of bacteria, classifiable as the " sickness " group.

The investigations have demonstrated that individual ciders differ considerably in degree of resistance to the disorder, and a study of the factors concerned has been undertaken.

Apart from the influence of the sugar and acid contents that of other constituents of the liquor, affecting its quality as a nutrient medium for the sickness organisms, is all-important. The identity of these substances is still under examination, although it is clear that nitrogen-containing organic compounds must be included among those concerned. It is probable that the quantity of any of them required for sufficient growth of the bacteria to induce definite sickness symptoms is very small. One or more of the " trace " elements may have to come into consideration—including molybdenum, which now has been shown definitely to stimulate growth-activity.

The effects of exposing the liquor to different temperatures above 45° C. for five minutes are also suggestive ; for, while at ranges between 50° C. and 75° C. a change is brought about in the liquor rendering it immune to infection, a further change occurs in some ciders heated to 80—100° C. making them able to support some growth of the organisms, especially if the ciders are neutralised with $CaCO_3$ before receiving the heat treatment. These results seems to exclude such enzymes as may be present in cider from any essential nutritional part in the development of the organisms in the heated liquor, whatever part they may play in the unheated beverage.

Reviewing the available evidence as a whole, the position appears to be that " sickness " bacteria are of widespread distribution and of common, if not universal, occurrence in expressed apple juices and ciders. In these they come into competition with naturally-occurring yeasts and bacteria of various kinds and the result of the conflict in any individual cider depends upon the joint effects of the chemical composition of the liquor and the conditions and treatments to which it is subjected. The conditions favouring the sickness organisms in the contest are low acidity and low nitrogen content of the ciders, the latter restraining in particular healthy and free growth of the yeasts.

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# THE RÔLE OF NITROGEN IN FERMENTATION. 1. CHANGES IN THE NITROGEN CONTENT OF APPLE JUICE DURING FERMENTATION.

By S. W. CHALLINOR and L. F. BURROUGHS.

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## INTRODUCTION.

When apple juice is fermented the quality of the resulting cider depends (i) upon the raw material, i.e. the juice, and (ii) upon the yeasts present in that juice. These two factors mainly determine the course of the fermentation. The importance of starting with suitable raw material has been amply demonstrated by the numerous cider-making trials, on single varieties of apples, which have been carried out at this Research Station by Prof. Barker and his colleagues. Barker has also shown that many different varieties of wild yeasts are present in the juice from the pressing of a single variety of fruit (unpublished observations) and that different types of yeast predominate at various stages of the fermentation (1).

In this paper the course of the fermentation of apple juice is studied in relation to one of its most important constituents—nitrogen. This element is one of the main nutritional requirements of yeast and is of fundamental importance in fermentation.

It is only possible here to mention briefly the more important conclusions of previous work concerning the relation of nitrogen to fermentation in cider-making.

The analytical data presented in the reports of the cider-making trials, to which reference has been made (see also Steuart (2)) show that many of the juices used in cider-making have a very low nitrogen content as compared with juices of culinary and dessert apples, and that the nitrogen content of different juices varies widely, from 3-15 mg. per 100 ml. of juice. The trials have also demonstrated that the rate of fermentation is related to the nitrogen content of the juice. This latter relationship has more recently been examined by the present authors (3).

*Removal of nitrogen* from the juice as a means of controlling fermentation has received considerable attention in the past. Thus, in the process of defecation, which is still practised extensively in France (4), nitrogenous compounds are removed by precipitation prior to the main fermentation. In the paper "Fermentation Control of Ciders by the Centrifuge Method," Charley (5) has shown that centrifugation of a fermenting juice removes nitrogen in the form of yeast and provides a method of checking the fermentation of the juice. In that paper (5) the relationship between changes in nitrogen content and the subsequent rates of fermentation is studied. Further reference to this method of control of fermentation by the centrifuge is made in the following paper (6) in this Annual Report in which the mechanism of the method is discussed.

The two methods of checking fermentation mentioned above, in which nitrogen plays an important part, are of considerable practical importance to the cider maker who is thereby enabled to produce naturally sweet ciders. A final reference to the importance of nitrogen in cider-making may be made, namely the influence of the nitrogen present in the finished product on the *stability* of the cider. In bottled cider, the amount of fermentation which can be brought about by any yeasts which may be present largely depends upon the amount of the residual nitrogen in the cider and upon its availability to the yeasts.

The main object of the work described in this paper was the observation of the changes in the nitrogen content of a juice and the associated changes in specific gravity which occurred when the juice was allowed to ferment naturally i.e. without any disturbance by centrifuging or any other process. From the observed changes in "total" and in "soluble" nitrogen the *course*

*of the growth* of yeast in the fermenting juice was determined and the *distribution* of the yeast—yeast in suspension, yeast in the lees—was ascertained. Some observations on autolysis were made.

Three fermentations were studied in detail : (1) fermentation of a relatively large volume (110-120 gallons) of juice in a pipe under cider-house conditions, (2) fermentation of a relatively small volume ($2\frac{1}{4}$ gallons) of juice in a glass jar under the same conditions as for (1), and (3) fermentation of the juice in a glass jar ($2\frac{1}{4}$ gallons) at 25° C. The experimental procedure for these *three* fermentations is described in the section headed " EXPERIMENTAL." It has been found convenient to divide " RESULTS AND DISCUSSION into two parts : PART I relating to the fermentation in the pipe : PART II relating to the fermentations in the glass jars (see Table of Contents). Comparisons between the three fermentations are made in the GENERAL DISCUSSION.

The choice of the juice (Bramley) used in these experiments was governed by practical considerations. It was hoped that the results of this investigation might eventually lead to explanations of the mechanisms of cider-making-procedures such as the " Fermentation Control of Ciders by the Centrifuge Method," the mechanism of which is still not fully understood. With this object in view an experiment in which the fermentation of the juice was checked by centrifuging at different stages in the fermentation was carried out at the same time as the experiments described in this paper. It was necessary to use the same juice for these two investigations and the Bramley juice was the only juice available at the time in the quantity required. The results of the " checked fermentation " experiment are described in the following paper (6).

## EXPERIMENTAL.

*Processing and Sampling Procedure.* Approximately seven tons of cull apples, of which 90-95% was Bramley's Seedling, were milled and pressed on the 10th and 11th of November 1948 and the fresh juice was bulked in a large vat and then circulated (by means of a pump) for two hours in order to ensure thorough mixing. The bulked juice was transferred (November 12th) into a series of eleven pipes (labelled A to K) of which seven (labelled A-G) were used for the work described in this and the following paper, and also into two glass jars each of $2\frac{1}{4}$ gallon capacity. The pipes (each of 110-120 gallon capacity) had previously been carefully cleaned and steamed out twice on consecutive days. Owing to lack of space in the cider-house itself the pipes were situated outside, in the cider-house yard. The fermentation of the juice in *one* of these pipes (F) was investigated fully as described in this paper. Some observations on autolysis which occurred in the juice fermented in pipe E were also made. One of the glass jars was placed adjacent to the pipes (i.e. in the cider-house yard) and the other was incubated at 25° C. (77° F.).

The manner in which the bulked juice (thoroughly mixed as described above) was distributed into the pipes (and jars) was as follows : Pipes A to G were filled in the order of lettering, then the other four pipes and the glass jars were filled with the remainder of the juice. Pipe F was therefore the sixth (middle) pipe to be filled and the jars were filled after pipe F, but not immediately afterwards.

In spite of the precautions taken to ensure uniformity the juices in the different fermentation vessels would be expected to show some differences. The extent of these differences is indicated by the following observations : There

was some variation in the nitrogen contents of the juice in the pipes and jar. The initial "total nitrogen" and initial "soluble nitrogen" (see later) in the juice in three of the pipes and in one of the glass jars were as follows :

| Pipe | "Total Nitrogen" | "Soluble Nitrogen" |
|---|---|---|
| C .. .. | 9·0 | 8·0 |
| F .. .. .. | 8·5 | 7·7 |
| I .. .. .. | 8·8 | 7·7 |
| Glass Jar .. .. | 8·9 | 7·5 |
| Average .. .. | 8·8 | 7·7 |

Specific gravity changes during fermentation in these pipes were observed and the fermentations were closely similar except for pipe A, in which the juice fermented rather more quickly than in the other pipes.

In pipe F the fermentation was allowed to proceed undisturbed, and small samples, approximately 220 ml. each, were simultaneously withdrawn at frequent intervals from the centre of the pipe, and from the glass jar in the cider-house yard, for nitrogen determinations ; specific gravities were also determined on these samples (after degassing) and were corrected for temperature. When the specific gravity of the cider in the pipe had ceased to fall (S.G. $1002\frac{1}{2}$), a sample was taken and the cider was then centrifuged through the Sharples Centrifuge at the rate of 70 gallons per hour. The bulked centrifuged cider was sampled and then stored in a smaller pipe in the cider-house. After storage for about three months a final sample was taken.

The juice in the glass jar in the incubator (25° C.) fermented more rapidly than that exposed to the lower temperature of the cider-house yard and was sampled at shorter intervals.

*Determination of Nitrogen.* The "total nitrogen" of each sample of juice or cider was determined on the sample direct. A part of the sample was centrifuged in the laboratory at 2,500 r.p.m. for 30 minutes and the "soluble nitrogen" determined on the supernatant ; the single sample from the Sharples centrifuge was not further centrifuged.

Special care was given to the determination of nitrogen. These analyses are complicated by the presence in the ciders of relatively large amounts of organic matter and only very small amounts of nitrogen.

Nitrogen was determined on the micro-scale by the Kjeldahl method : the sample (10 ml.) was acidified with $H_2SO_4$ and evaporated to a black syrup in the presence of 0·8 ml. $H_2O_2$ (100 vol.). After a further addition of acid (3 or 4 ml. depending upon the S.G. of the sample) the catalyst (0·5 g.) as described by Chibnall *et al* (7) was added and the digestion continued until the digest became clear and green. After cooling, a further addition of $H_2O_2$ (0·2 ml.) was made and the digestion continued for half an hour. Digests were made up to a volume of 50 ml. and duplicate aliquots (10 ml.) were separately distilled (Markham apparatus) into 0·5% boric acid (2 ml.) and titrated with $N/_{140}$ $H_2SO_4$ from a 1 ml. burette. Titrations ranged from 1·5 ml.—0·3 ml. of the acid and it was possible to determine the endpoint to within 0·01 ml. or less.

The digestion procedure adopted is more suitable than the 8-hour digestion period (no addition of $H_2O_2$) when large numbers of analyses are required. A comparison of the two methods of digestion was made on three of the samples. The results are tabulated below and indicate that recovery of nitrogen by the shorter digestion period was complete.

| Sample | mg.N/100 ml. sample | | | |
|---|---|---|---|---|
| | 8 hours digestion | | shorter digestion | |
| 1. | 8·6<br>8·6<br>8·6 | 8·6 | 8·2<br>8·4<br>8·6 | 8·4 |
| 2. | 7·0<br>7·0<br>7·0 | 7·0 | 7·0<br>7·1<br>7·2 | 7·1 |
| 3. | 5·2<br>5·1<br>5·4 | 5·2 | 5·1<br>5·1<br>5·0 | 5·1 |

## RESULTS AND DISCUSSION.

## PART I. THE FERMENTATION OF THE JUICE IN PIPE F AT OUTDOOR TEMPERATURES.

In this first part of the Results and Discussion consideration will be given to the fermentation in the pipe F, at outdoor temperatures, and to the accompanying changes in the nitrogen content of the fermenting juice. A description of the fermentations and changes in nitrogen which took place in the juice in the two glass jars will be given in the second part of this discussion.

### (1) Changes in Specific Gravity During the Fermentation.

The fermentation was rather slower than expected for a juice of the Bramley variety, even when allowance was made for the fact that the fermentation was taking place at outdoor temperatures, but this is in agreement with the nitrogen content of the juice (8·5 mg.N/100 ml.) which is low for the variety. As indicated in Graph Ia there was a lag period of three days during which little decrease in specific gravity occurred. In this connexion, the interval of time (two days) between the start of the milling and pressing operations and the distribution of the bulked juice into the separate pipes and jars has also to be considered—the lag period therefore extended over three to five days although only three days are indicated on the graph. Fermentation then became more rapid and the rate was approximately 2 degrees decrease in S.G. per day. Between the 10th and 25th days the rate of fermentation was slower and was sensibly constant over the period (approximately 1 degree decrease in S.G. per day). The rate of fermentation then became progressively slower and only seven degrees of specific gravity were lost during the next fifteen days, the S.G. on the 39th day being 1005. Subsequent fermentation was extremely slow and only $2\frac{1}{2}$ degrees of specific gravity were lost during the next thirty-five days. At seventy-four days the S.G. was $1002\frac{1}{2}$ and the cider was centrifuged.

Temperature observations during the fermentation period were as follows : The mean daily temperature for *each* of the first twenty-five days of the fermentation period ranged from 39° F. to 56° F. and averaged 48° F. During the remainder of the period rather lower temperatures occurred (range 27° F. to 54° F., average 42° F.) and the average of the mean daily temperatures, over the whole period of seventy-four days, was 44° F.

**(2) Changes in Nitrogen during the Fermentation in Pipe F and Conversion of Nitrogen-Data into Data Representative of Yeast.**

In the introductory paragraphs mention has been made of the different aspects of the nitrogen content of the fermenting juice which are considered here in relation to fermentation, namely : (a) " total nitrogen," (b) " nitrogen in solution," (c) " nitrogen in suspension," (d) " nitrogen in the lees," (e) growth of yeast during the fermentation. These aspects of the nitrogen content of the fermenting juice will be considered separately but it is necessary first to describe more fully the sampling procedure and then to explain the method by which the nitrogen-data is converted into data representative of yeast e.g. yeast growth, yeast in suspension and yeast in the lees—and to indicate the limitations of this method of conversion.

The *period of two days* which elapsed between the *start* of the pressing operations and the first sampling of the juice will be referred to as the " processing period " and it should be emphasized that it is not included in any of the time-data in the text, tables or graphs. Withdrawal of samples from the pipe and glass jars was carried out in as uniform a manner as possible, from the middle (approximately) of the fermentation vessel and without disturbing the lees. The *samples* were, therefore, representative of the fermenting juice but *not inclusive of the lees.* There was no appreciable amount of lees in the glass jars at the *start* of the experiments and this was presumably true also for the pipe. It was not possible to carry out microbiological observations throughout these investigations but such observations were made on some of the early samples. The solid material in suspension in the first samples of the juice from the pipe and glass jars consisted mainly of yeast ; only relatively small numbers of yeast cells were present.

The method of conversion of nitrogen-data into data representative of yeast will now be explained. The explanation is applicable to the interpretation of the data relating to each of the three fermentations. It must be emphasized that no attempt has been made to determine the *absolute amounts of yeast* present at different stages of the fermentations. The relative amounts of " *nitrogen* " present at different times in the fermenting juices *which could be regarded as yeast-nitrogen* (see below), are taken as representative of the relative amounts of *yeast* present at those times. *All analyses and calculations refer to* 100 *ml. of fermenting juice or cider.*

The " fresh " juice, i.e. after the " processing period," contained little suspended solid material and, since (as stated above), this was mainly yeast, it is reasonable to regard the nitrogen content of this suspended material, i.e. " nitrogen in suspension," as indicative of the amount of *yeast* in suspension. The amount of yeast in suspension varied during the fermentation and the *relative amounts of " nitrogen in suspension "* in the fermenting juice at different times are taken as *representative of* the *relative amounts of yeast in suspension* at those times.

The course of yeast growth in the fermenting juice was followed by observations on the " soluble nitrogen " content of the juice. Thus, the amount by which " nitrogen in solution " decreased in a given time is taken as indicative of the amount of yeast which had grown in that time. This is justifiable provided that the decrease in the " soluble nitrogen " content of the fermenting juice was not due to any factor other than growth of yeast. Two such factors need to be considered : (1) loss of " nitrogen " in the form of volatile products—extremely unlikely from a fermenting apple juice, (2) conversion of soluble nitrogenous compounds into insoluble material other than yeast—this is not likely to occur.

It is necessary also to consider possible *increases* in " soluble nitrogen " e.g. due to autolysis which did in fact occur in each of the fermentations. Such an increase would result in the calculated values for amount of " yeast which has grown " being *smaller* than such values calculated before the increase in " soluble nitrogen " occurred. This explains the apparent decrease in the amount of yeast growth recorded in the later stages of each of the three fermentations. This effect was most marked in the fermentation at 25° C.

It must be emphasized that there is a difference between the amount of yeast *present* at any time (i.e. in the lees and in suspension) and the amount of yeast which has *grown* during the period from the start of the experiment up to that time. This difference is the amount of yeast which was already present (in suspension) at the start of the experiment.

The method by which the relative amounts of " nitrogen in the lees " at different times during the fermentation are determined is explained in the appropriate section. These " *lees-nitrogen* " contents are taken as indicative of the relative amounts of *yeast in the lees.* Non-nitrogenous material, such as pectin degradation products, will also be present in the lees at certain stages of the fermentation (possibly also in suspension) but this does not affect the way in which the nitrogen-data are interpreted.

It is realised that the above treatment of the nitrogen-data is subject to certain limitations (that due to autolysis has already been mentioned). Thus, different varieties of yeasts are usually present in fermenting apple juice and the type of yeast which predominates, for example, in the early stages of the fermentation, differs from that which predominates in the later stages. The nitrogen contents of different yeasts are, however, variable and the factor for converting " yeast-nitrogen " into yeast may therefore vary with the yeast. Also, the method used for determination of nitrogen (Kjeldahl) is not capable of determining *all* the nitrogen present in certain types of nitrogenous compounds, e.g. pyrimidines and purines. In attempting to assess the importance of these limitations, it must be borne in mind that the derived-data for yeast is used in a *comparative* manner, e.g. the *relative* amounts of yeast in suspension at different times during the fermentation, the *relative* amount of yeast in suspension as compared with the total amount of yeast present, etc. Furthermore, these methods easily bear comparison with microbiological methods of determining the numbers of yeast cells in a fermenting juice, since such methods are subject to considerable experimental error. The latter methods, however, have the advantage of distinguishing between *living* and *dead* yeasts, a distinction which is not possible by the methods under consideration.

Before proceeding to the discussion of the nitrogen changes which occurred during the fermentations it is necessary to point out that, although samples were withdrawn from pipe F and from the glass jar adjacent to the pipe at frequent intervals up to the 25th day, there was a *long interval* between this sampling and the final sampling which was taken on the 74th day. The specific gravity of the cider in pipe F (only) was taken on the 39th day but nitrogen was not determined at this time.

(a) " *Total Nitrogen.*" By this is meant the summation of the nitrogenous compounds in solution and of the material in suspension (mainly or entirely yeast). " Total nitrogen " was determined on the samples direct, i.e. without centrifuging ; each sample was thoroughly shaken before analysis. As already stated " total nitrogen " *does not include any* " *lees-nitrogen* " (*lees-yeast*) ; this was left in the pipe and increased in amount as the experiment progressed. During the fermentation, " total nitrogen " decreased in amount from 8·5—1·8

## TABLE I.

### Fermentation in Pipe F at Outdoor Temperatures.

Changes in the Nitrogen Content of the Fermenting Juice. Table also gives Values (Calculated from the Nitrogen-Data) Representative of the Growth and Distribution of Yeast in the Fermenting Juice. (All nitrogen contents are expressed as mg.N/100 ml. fermenting juice or cider).

| Days of Fermentation | Specific Gravity | Nitrogen in the Fermenting Juice | | "Yeast-Nitrogen" | | | |
|---|---|---|---|---|---|---|---|
| | | "Total Nitrogen" | "Nitrogen in Solution" | "*Total Nitrogen*" minus "*Soluble Nitrogen*" i.e. "*Nitrogen* in *Suspension*" Representative of *Yeast in Suspension* | *Decrease in* "*Total Nitrogen*" Representative of nitrogen in the "lees" and of *Yeast in the Lees* | *Decrease in* "*Soluble Nitrogen*" Representative of *Growth of Yeast* | "*Initial Total Nitrogen*" minus "*Soluble Nitrogen*" Representative of *Total Yeast Present** |
| 0 | 1,046½ | 8·5 | 7·7 | 0·8† | 0·0 | 0·0 | 0·8† |
| 1 | 1,045½ | 7·8 | 7·3 | 0·5 | 0·7 | 0·4 | 1·2 |
| 2 | 1,045 | 7·1 | 6·2 | 0·9 | 1·4 | 1·5 | 2·3 |
| 3 | 1,044 | 6·3 | 5·1 | 1·2 | 2·2 | 2·6 | 3·4 |
| 4 | 1,040 | 4·6 | 3·0 | 1·6 | 3·9 | 4·7 | 5·5 |
| 6 | 1,035½ | 3·4 | 1·7 | 1·7 | 5·1 | 6·0 | 6·8 |
| 8 | 1,031½ | — | 1·5‡ | — | — | 6·2 | 7·0 |
| 10 | 1,028 | 2·7 | 1·5‡ | 1·2 | 5·8 | 6·2 | 7·0 |
| 15 | 1,022½ | 2·5 | 1·5‡ | 1·0 | 6·0 | 6·2 | 7·0 |
| 18 | 1,019 | 2·1 | 1·5‡ | 0·6 | 6·4 | 6·2 | 7·0 |
| 25 | 1,012 | 2·1 | 1·4F | 0·7 | 6·4 | 6·3 | 7·1 |
| 74 | 1,002½ | 1·8 | 1·8F | 0·0 | 6·7 | 5·9 | 6·7 |

‡ Value to which the "soluble nitrogen" falls after utilisation by the yeasts of the readily available soluble nitrogenous compounds of the juice.
F Filtered sample (Centrifuge not available).
* "*Total Yeast Present*" at any time is that present at the start of the experiment (proportional to 0·8 mg.N/100 ml.) plus the yeast which has grown (see preceding column) up to that time.
† This value, 0·8 mg.N/100 ml., is representative of the amount of yeast present (in suspension) in the juice at the start of the experiment.

mg.N/100 ml. of fermenting juice, i.e. to 21% of its initial value (Table I). *Fig.* 1a shows that the greater part of this decrease (8·5—3 mg.N/100 ml.) took place rapidly and at an approximately constant rate. This decrease, 5·5 mg.N/100 ml., represents 4/5th of the over-all decrease in " total nitrogen " and took place during the first seven to eight days of the fermentation ; it was associated with a fall in specific gravity of only 14 degrees ($1046\frac{1}{2}$—$1032\frac{1}{2}$) which represents approximately 1/3rd of the fall in S.G. which occurred during the whole fermentation (44 degrees). The remaining 1/5th of the decrease in " total nitrogen," 3—1·8 mg.N/100 ml., associated with the latter 2/3rd

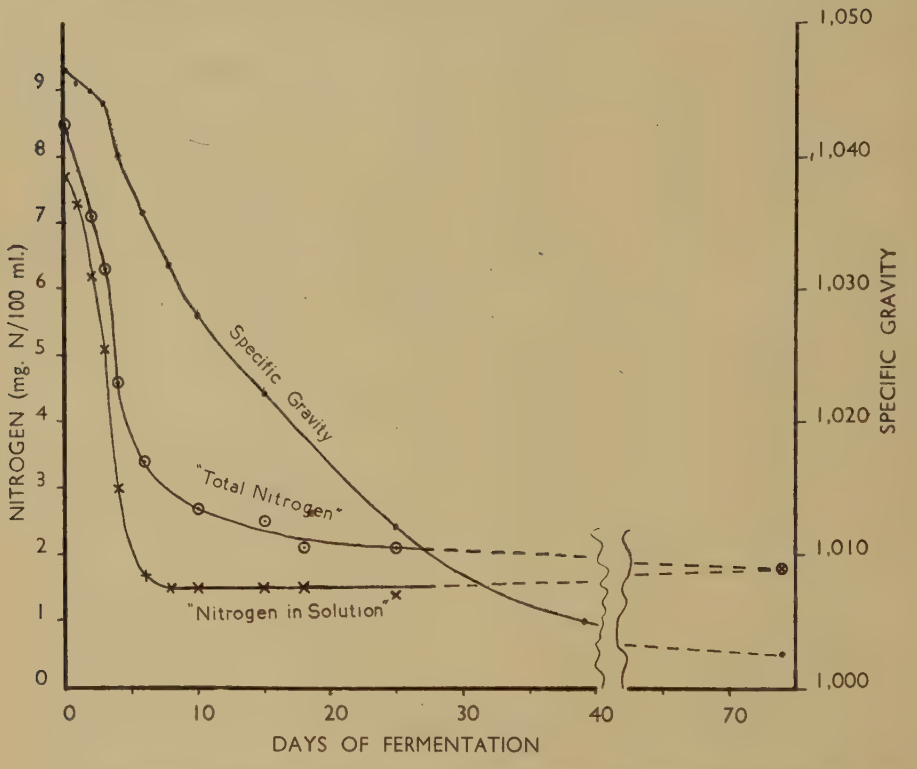


*Fig.* 1a. Fermentation in Pipe F at Outdoor Temperatures. Specific Gravity Curve and Changes in "Total Nitrogen" and in "Nitrogen in Solution" of the Fermenting Juice.

of the fermentation (30 degrees fall in specific gravity) occurred during a much longer period of time ; most of this decrease (75%) was associated with a fall in S.G. of 20 degrees and the final 10 degrees loss in S.G. took place with very little decrease in the " total nitrogen " (0·3 mg.N/100 ml.).

(b) " *Nitrogen in Solution.*" This is the nitrogen remaining in the sample after centrifuging at 2,500 r.p.m. for thirty minutes on the laboratory centrifuge —a treatment which removed the " nitrogen in suspension " (yeast). It is more fundamental than " total nitrogen " which includes the yeast in suspension, the amount of which is determined by unknown factors which control the course and vigour of the fermentation. As will be seen from *Fig.* 1a and Table I the relationship between " soluble nitrogen " and time is similar to that between " total nitrogen " and time. During the first seven to eight days of the fermentation the decrease in " soluble nitrogen " was greater than, and largely accounted for, the corresponding decrease in " total nitrogen."

There was an *immediate* and rapid decrease in the amount of " soluble nitrogen " although, in this connexion the two day " processing period," which occurred prior to the start of the experimental observations, should be borne in mind. By the 3rd day, approximately half of the " soluble nitrogen " which was available to the yeasts had been utilised. Reference to *Fig.* 1a shows that the specific gravity curve has a point of inflexion on the 3rd day, the rate of fermentation increasing greatly at about that time. Thus, the losses in specific gravity during the first three days and the subsequ nt three days are $2\frac{1}{2}$ and $8\frac{1}{2}$ degrees respectively. It would appear, therefore, that approximately half of the " soluble nitrogen " which was utilised by the yeasts during the whole of the fermentation, was utilised *before* the fermentation became vigorous.

" Soluble nitrogen " decreased in amount from 7·7—1·5 mg.N/100 ml. of fermenting juice, i.e. to 19·5% of its initial value ; the whole of this decrease occurred during the first seven to eight days of fermentation. Most of this decrease was rapid and at an almost constant rate ; it was accompanied by a loss of only 14 degrees in specific gravity which corresponded to approximately one third of the total decrease in S.G. The remaining *two-thirds of the fermentation* (30 degrees S.G.) *was not accompanied by any observable decrease in the " soluble nitrogen " of the fermenting juice.* In fact, an *increase* was observed in the " soluble nitrogen " after the 25th day (from 1·5—1·8 mg.N/100ml.) and this was presumably due to autolysis (see later).

It has been stated above that the " soluble nitrogen " decreased in amount to 19·5% of its initial value ; this indicates that about 80% *of the soluble nitrogenous compounds in the juice was in a form easily available to the yeasts.*

(c) " *Nitrogen in Suspension.*" This is the *nitrogen* of the material suspended in the fermenting juice and, as explained in the beginning of this section, may be considered as representative of the *yeast* in suspension. It was not determined directly but was obtained by subtracting the " soluble nitrogen," i.e. nitrogen on the centrifuged sample, from the " total nitrogen " as determined directly on the sample before centrifuging. The vertical distance between the curves for " total nitrogen " and for " soluble nitrogen " (*Fig.* 1a) is representative of the " nitrogen in suspension " at any time ; this relationship is however more clearly shown in *Fig.* 1b.

Before discussing the changes which occurred in the amount of " nitrogen in suspension " it is necessary to refer to the small amount of lees which was probably present in the bulked juice in the vat from which the pipes were filled. During the mixing of the juice and its transference into the pipes this lees material would be brought into suspension. The value for " nitrogen in suspension " at the start of the experiment is, therefore, likely to be higher than it would otherwise have been. Little significance can, therefore, be attached to the decrease in the amount of " nitrogen in suspension " which was observed during the 1st day. After this decrease there was a rapid and steady increase in the amount of " nitrogen in suspension " (yeast) and it had attained approximately three quarters of its peak value on the 3rd day, when approximately half the available " soluble nitrogen " had been utilised. At this time the rate of fermentation increased greatly (rate of fermentation was at its maximum between the 3rd and 4th days). Yeast in suspension reached its peak, 1·7 mg.N/100 ml. of fermenting juice, in about five days (during which time 9 degrees of specific gravity were lost) and remained at or near the peak value during the period four to eight days. The peak value

was reached before the " soluble nitrogen " had reached its minimum. Comparison of the amount of yeast in suspension when at its maximum and that present in the lees (discussed later) at that time, shows that the amount of yeast in suspension was about one third of that in the lees. Between the 6th and 8th days the amount of yeast in suspension began to fall although " soluble nitrogen " was still slowly decreasing in amount, i.e. was still being utilised (yeast was still growing).

The decrease in the amount of yeast in suspension, due to sedimentation, continued progressively over a fairly long period. Rate of fermentation

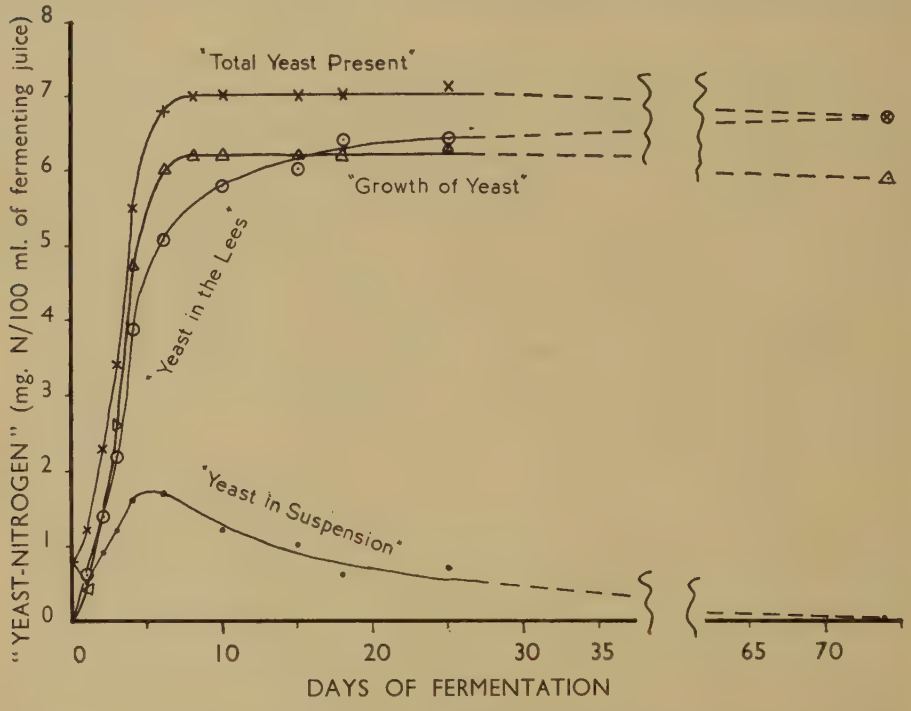


*Fig.* 1b. Fermentation in the Pipe F at Outdoor Temperatures, showing the Growth and Distribution of " Yeast " in the Fermenting Juice.

N.B. The ordinates in *Fig.* 1b represent " yeast-nitrogen." These values are taken as representative of yeast (see text)

decreased appreciably about the 10th day but was approximately constant between the 10th and 25th days, although yeast in suspension was decreasing markedly during this period. Sedimentation of the yeast was appreciably slower during the period fifteen to twenty-five days than during the period eight to fifteen days. On the 25th day the " nitrogen in suspension " was 0·6 mg.N/100 ml. i.e. the *yeast* in suspension was about one third of its peak value. On the 74th day there was no yeast in suspension (or an insignificant amount). Yeast in suspension increased in amount during the first third of the fermentation and decreased during the latter two thirds of the fermentation.

Growth of yeast during the fermentation is discussed later but it is convenient to make some comparison at this stage between yeast in suspension and total yeast growth. Thus, the rapid increase in the amount of yeast in suspension which occurred during the early stages of fermentation corresponded closely with the rapid growth of yeast which took place during those stages—

and the amount of yeast in suspension started to decrease when the growth of the yeast ceased or became very slow (after eight days).

(d) " *Nitrogen in the Lees.*" The amount of nitrogen in the yeast which sedimented out to form the lees was not determined directly. As the fermentation progressed yeast sedimented into the lees and, since all samples of the fermenting juice were withdrawn without disturbing the lees, the amount of " total nitrogen " in these samples became progressively smaller. The *decrease in " total nitrogen "* which occurred during any period of the fermentation was representative of the amount of nitrogen, and therefore of yeast, which was deposited into the lees during that period. " Nitrogen in the lees " at any time during the fermentation was therefore obtained by subtracting the " total nitrogen " (" nitrogen in suspension " plus " nitrogen in solution ") of the juice at that time from the *initial* " total nitrogen " of the juice (8·5 mg.N/100 ml.) at the start of the experiment. It follows, that the smaller the " total nitrogen " at any time, the greater will be the " lees-nitrogen " at that time and vice-versa. Since the experimental observations started two days after the commencement of the milling of the fruit, it is possible that a very small amount of lees might have been present in the pipe at the start of the experiment but any such lees can be disregarded without materially affecting the results of the experiments. From the method of calculating the " lees-nitrogen " as indicated above, it follows that the results refer to that amount of lees-nitrogen which was formed from 100 *ml. of original juice* and which was present at the time the samples were taken.

The amount of nitrogen and therefore of yeast in the lees increased very rapidly during the first five to six days of fermentation (*Fig.* 1b). At this time. the amount of yeast in the lees was about three times as great as the amount of yeast in suspension (then at its peak) as indicated by the nitrogen-data. Yeast in the lees continued to increase in amount but after the 20th day the rate of increase was very slow. During the period five to eight days, yeast in the lees increased at a greater rate than yeast in suspension decreased, (appreciable growth of yeast was still taking place). After the 8th day any increase in the amount of yeast in the lees could be directly accounted for by the decrease in the amount of yeast in suspension, i.e. the amount of yeast which sedimented out, indicating that yeast growth had practically ceased at that time. No decrease in the amount of lees was observed during the latter part of the fermentation, and since autolysis did in fact occur, it would appear that any loss in the lees-yeast due to autolysis was more than balanced by the suspended yeast which sedimented out, although sedimentation was very slight at this stage of the fermentation.

(e) *Growth of Yeast During the Fermentation and " Total Yeast Present."*

The difference between the *initial* " soluble nitrogen " of the fermenting juice (7·7 mg.N/100 ml.) and the " soluble nitrogen " at any time during the fermentation (i.e. decrease in " soluble nitrogen ") was taken as indicative of the amount of yeast growth at that time and on this basis the growth curve was plotted (*Fig.* 1b). It follows that the smaller the " soluble nitrogen " at any time the greater will be the calculated yeast growth at that time and vice-versa. No very definite " lag " phase is shown but in this connexion it must be remembered that the " processing time " prior to experimental observations (two days) is not indicated on the graph. Growth of yeast was very rapid and had reached 40% of completion in only three days (at this time the rate of fermentation increased greatly). During the period six to eight days the rate of growth of yeast decreased progressively and growth

had practically ceased by the 8th day. During this first eight days 15 degrees of specific gravity were lost, i.e. about one third of the loss in specific gravity which occurred during the whole of the fermentation. During the period eight to twenty-five days there was apparently no further growth of yeast and there was no significant change in the rate of fermentation. An appreciably smaller amount of yeast was *present* on the 74th day, presumbly due to autolysis.

As stated earlier in this section there is a difference between the amount of yeast *present* at any time (i.e. in the lees and in suspension) and the amount of yeast which has *grown* during the period from the start of the experiment up to that time. This difference is the amount of yeast which was already present (in suspension) at the start of the experiment (equivalent to 0·8 mg.N/100 ml.) The amount of yeast present at any time during the fermentation is graphed as " total yeast present." (*Fig.* 1b) (see also Table I).

**(3) Autolysis.**

The cider in pipe F had a " soluble nitrogen " content of 1·5 mg.N/100 ml. (*Fig.* 1a) on the 25th day and 1·8 mg.N/100 ml. on the 74th day. Prior to the 25th day, the " soluble nitrogen " had remained substantially constant (analyses on four consecutive samples, Table I, *Fig.* 1a) and the increase mentioned above, 0·3 mg.N/100 ml., indicated appreciable autolysis. This increase in " soluble nitrogen " is, of course, responsible for the decrease in the value calculated for " total yeast present " which occurred during this period and to which reference has been made in the last paragraph. The increase in " soluble nitrogen " represents 4% of its initial value and 20% of its minimum (1·5 mg.N/100 ml.) and the extent of autolysis indicated is of considerable interest. The amount of yeast which was autolysed may be estimated approximately as follows: The increase in " soluble nitrogen," 0·3 mg.N/100 ml., corresponds to approximately 1·9 mg. of protein i.e. approximately 3·8 mg. dryweight of yeast, if it is assumed that all the increase in " soluble nitrogen " is derived from yeast and that the average protein content of cider-yeasts is of the order of 50%. On this basis the above calculation would give a *minimum* value for yeast autolysed since not all the nitrogenous products formed as a result of autolysis of yeast would be soluble. On the 25th day the " total yeast present " per 100 ml. of cider (calculated from the nitrogen-data) was 88 mg. It would appear, therefore, that approximately 4% or more of this yeast had been autolysed at some time during the period 25th to 74th days of fermentation.

During the period of time under discussion, 25th—74th days, the specific gravity decreased by $9\frac{1}{2}$ degrees. Precise information of the time interval during which autolysis occurred is not available for reasons previously stated.

A greater extent of autolysis, as indicated by the increase in " soluble nitrogen," was recorded (over a longer period of time) in the experiment on the cider in *pipe E*. This cider had been allowed to *stand on its lees* for three months after fermentation had ceased (three months after the 74th day at which time the specific gravity had fallen to $1002\frac{1}{2}$). On the 74th day the " soluble nitrogen " in the cider was 1·8 mg.N/100 ml. and was in close agreement with the corresponding " soluble nitrogen " in the cider of pipe F (and also in another pipe D, see following paper) at that time. It is to be expected, therefore, that the cider in pipe E already contained (i.e. on the 74th day) autolytic products of the same order in amount as the cider in pipe F, since both pipes had been treated in the same way until the 74th day. During the

three months *storage* on the lees the " soluble nitrogen " in the cider in pipe E further increased to 2·3 mg.N/100 ml.—an increase of 0·5 mg.N/100 ml. This increase is equivalent to 28% of the " soluble nitrogen " present (1·8 mg.N/100 ml.) prior to the three months standing on the lees and indicates additional autolysis.

Further observations were made on this cider in pipe E. At the end of the three months standing on the lees, samples were taken at three different levels in the pipe and also from the bottom of the pipe (i.e. a sample of the lees) and the " total " and " soluble " nitrogen contents were determined ; the results are tabulated below.

| Depth in pipe at which the Sample was withdrawn. | Nitrogen (mg./100 ml.) | |
|---|---|---|
| | " Total " | " Soluble " |
| Approx. 4″ from top | 2·3 | 2·2 |
| Middle | 2·3 | 2·3 |
| Approx. 6″ from bottom | 2·1 | 2·2 |
| In the lees | 79·0 | 3·8*<br>3·7* |

* " Soluble nitrogen " was determined on the samples after centrifuging at 2,500 p.m. for 30 mins. except for the lees-sample which was centrifuged at 2,500 (N = 3·8 mg./100 ml.) and at 4,000 r.p.m. (N = 3·7 mg./100 ml.).

The results, excluding the lees-sample, show that there was no " nitrogen gradient " and it would appear that the rate of distribution of the autolytic products throughout the pipe was greater than their rate of formation.

The centrifuging treatment which had proved effective in removing the suspended material from previous samples (prior to determination of " soluble nitrogen ") did not clear the lees-sample ; this sample was therefore centrifuged at a higher speed, 4,000 r.p.m., and was then moderately clear. The " soluble nitrogen " content of this sample was 3·7 mg.N/100 ml. i.e. twice as great as the minimum recorded for the cider in this pipe.

An attempt was made to ascertain the proportion of the solid material in *the lees-sample* which might be yeast. On this sample the " total nitrogen " was 79 mg.N/100 ml., the " soluble nitrogen " was approximately 4 mg.N/100 ml., and the difference, 75 mg.N/100 ml. was a measure of the amount of nitrogenous material in suspension ; this difference is equivalent to approximately 470 mg. of protein. The dry-weight of the material in suspension (dried over $P_2O_5$ in a desiccator) was 2·48 grams/100 ml. and the protein content of this solid material was therefore approximately 19%. If the protein content of dried cider-yeast is assumed to be of the order of 50% this data indicates that about 40% of the dry-weight of the insoluble solids in this lees-sample was yeast. The remainder of the insoluble solids in the lees would probably consist in part of pectin degradation products. These results are representative of the lees-sample, not of the lees as a whole.

## RESULTS AND DISCUSSION (Part II)

## THE FERMENTATION OF THE JUICE IN GLASS JARS.

The experimental procedure adopted for these fermentations was the same as that for pipe F and has been outlined in the EXPERIMENTAL section.

Owing to considerations of space it will not be possible to discuss these fermentations as fully as the fermentation in pipe F. The course of these fermentations and the accompanying nitrogen changes are clearly indicated in Tables II and III and in *Figs.* 2a, 2b, and 3a, 3b. In the following presentation and discussion of results emphasis is given to comparisons between : (A) the fermentation in the relatively small glass jar in the cider-house yard and that in pipe F, and (B) between the fermentations in the glass jar at 25° C. and in the glass jar in the cider-house yard.

The juice in the two glass jars and that in pipe F differed slightly in their nitrogen contents. A large volume of juice was handled in the experiments described in this and the subsequent paper and small differences in the amount of suspended yeast, for example, in the different containers is unavoidable. " Total " and " soluble nitrogen " of the juice in the pipe and glass jars were 8·5 and 7·7 mg.N/100 ml. in the pipe and 8·9 and 7·5 mg.N/100 ml. in the glass jars. Nitrogen determinations were not carried out on a sample of juice from each of the two jars, since the jars were filled from the same container.

Since the volume of juice in each of the glass jars was only $2\frac{1}{4}$ gallons (approximately) the reduction in volume due to removal of samples from the jars was significant, e.g. at the end of the fermentation the volume of cider was approximately $\frac{1}{3}$ –$\frac{1}{2}$ gallon less than the volume of juice at the start. This reduction in volume does not, however, materially affect the results obtained for " soluble " and " total nitrogen," nor the interpretation of these results, except when the interpretation of the extent of autolysis which has occurred is considered.

**A. The Fermentation in the Glass Jar at Outdoor Temperatures.**

The jar was situated in the cider-house yard near the pipe F. These two fermentation vessels differ (1) in the material of which they are made i.e. glass and wood respectively, (2) in capacity—the volume ($2\frac{1}{4}$ gallons) of the jar is relatively small compared with the 110-120 gallon capacity of the pipe, (3) in the relative air-spaces above the fermenting juice and in other ways e.g. shape. Because of the difference in material and volume, the response of the juice in these two vessels to changes of temperature would be different, the smaller volume of juice in the jar probably responding more quickly to changes of temperature than the juice in the pipe. This would also be expected from consideration of the materials of which the vessels are made—glass has a higher thermal conductivity than wood.

The average daily temperature ranged from 27° F. to 56° F. (—3° C. to 13° C.) during the fermentation ; further data re. temperature has been given in the description of the fermentation in pipe F.

This juice fermented rather more rapidly (*Fig.* 2a, Table II) than that in the pipe. For example, the sudden increase in rate of fermentation following the " lag " period occurred two days sooner (i.e. the " lag " period was shorter) and the specific gravities at corresponding times during active fermentation were 2-4 degrees lower in this juice than in the juice in the pipe. Apart from this difference, the two fermentations closely resembled each other. This is shown more clearly on *Fig.* 4 on which the three fermentation-curves (for the pipe and for each of the two glass jars) are plotted between the same axes for comparison. Thus, in the period between the end of the " lag " phase of growth of the yeast and the 25th day (twenty four days) 38 degrees of gravity were lost, i.e. approximately $1\frac{1}{2}$ degrees per day ; in the pipe 32 degrees of gravity were lost in the corresponding period (twenty two days) and this

## TABLE II.

### Fermentation in the Glass Jar at Outdoor Temperatures.

Changes in the Nitrogen Content of the Fermenting Juice. Table also gives Values (Calculated from the Nitrogen-Data) Representative of the Growth and Distribution of Yeast in the Fermenting Juice. (All nitrogen contents are expressed as mg.N/100 ml. fermenting juice or cider).

| Days of Fermentation | Specific Gravity | Nitrogen in the Fermenting Juice | | "Yeast-Nitrogen" | | | |
|---|---|---|---|---|---|---|---|
| | | "Total Nitrogen" | "Nitrogen in Solution" | "*Total Nitrogen*" minus "*Soluble Nitrogen*" i.e. "*Nitrogen in Suspension*" Representative of *Yeast in Suspension* | *Decrease in* "*Total Nitrogen*" Representative of nitrogen in the "lees" and of *Yeast in the Lees* | *Decrease in* "*Soluble Nitrogen*" Representative of *Growth of Yeast* | "*Initial Total Nitrogen*" minus "*Soluble Nitrogen*" Representative of *Total Yeast Present** |
| 0 | 1,046½ | 8·9 | 7·5 | 1·4† | 0·0 | 0·0 | 1·4† |
| 1 | 1,046 | 7·1 | 6·7 | 0·4 | 1·8 | 0·8 | 2·2 |
| 2 | 1,044 | 6·0 | 5·6 | 0·4 | 2·9 | 1·9 | 3·3 |
| 3 | 1,042 | 4·3 | 3·5 | 0·8 | 4·6 | 4·0 | 5·4 |
| 4 | 1,038 | 3·9§ | 2·2 | 1·7 | — | 5·3 | 6·7 |
| 6 | 1,035½ | 3·1 | 1·7 | 1·4 | 5·8 | 5·8 | 7·2 |
| 8 | 1,028½ | 2·5 | 1·4‡ | 1·1 | 6·4 | 6·1 | 7·5 |
| 10 | 1,025 | 2·2 | 1·4‡ | 0·8 | 6·7 | 6·1 | 7·5 |
| 15 | 1,019½ | 1·9 | 1·3‡ | 0·6 | 7·0 | 6·2 | 7·6 |
| 18 | 1,016 | 1·9 | 1·3‡ | 0·6 | 7·0 | 6·2 | 7·6 |
| 25 | 1,007½ | 1·8 | 1·2F | 0·6 | 7·1 | 6·3 | 7·7 |
| 74 | 1,001½ | 2·1 | 2·2F | 0·0 | 6·8 | 5·4 | 6·8 |

‡ Value to which the "soluble nitrogen" falls after utilisation by the yeasts of the readily available soluble nitrogenous compounds of the juice.
F Filtered Sample (Centrifuge not available).
* "*Total Yeast Present*" at any time is that present at the start of the experiment (proportional to 1·4 mg.N/100 ml.) plus the yeast which has grown (see preceding column) up to that time
† This value, 1·4 mg.N/100 ml., is representative of the amount of yeast present (in suspension) in the juice at the start of the experiment.
§ This value is interpolated from the graph, in order to calculate the peak value (1·7 mg.N/100 ml.) for "yeast nitrogen in suspension."

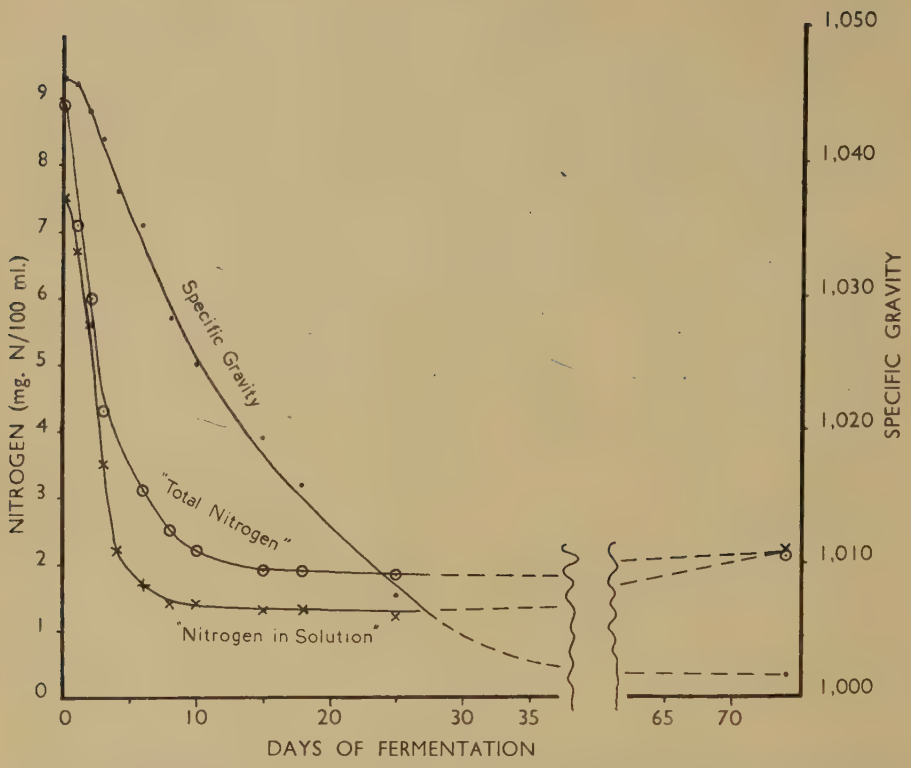


*Fig.* 2a. Fermentation in the Glass Jar at Outdoor Temperatures. Specific Gravity Curve and Changes in "Total Nitrogen" and in "Nitrogen in Solution" of the Fermenting Juice.

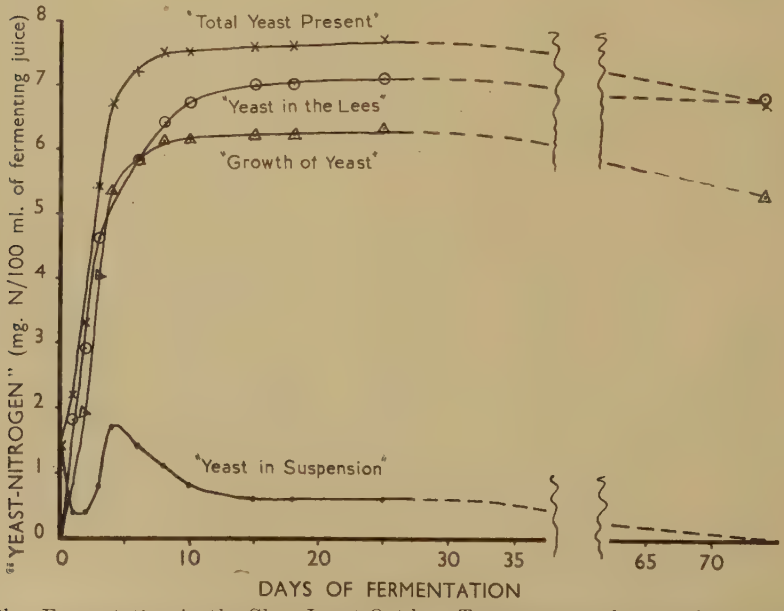


*Fig.* 2b. Fermentation in the Glass Jar at Outdoor Temperatures, showing the Growth and Distribution of "Yeast" in the Fermenting Juice.

N.B. The ordinates in *Fig.* 2b represent "yeast-nitrogen." These values are taken as representative of yeast (see text).

also represents a loss of 1½ degrees of gravity per day. Loss of specific gravity in the jar was 45 degrees (final S.G. 1001½) as compared with 44 degrees in the pipe. This greater loss in S.G. may have been related to the rather higher " total nitrogen " content of the juice in the jar.

Nine-tenths of the loss in " total nitrogen " which occurred during the whole of the fermentation, (c.f. four-fifths for the pipe) took place during the first eight days and the loss in gravity during this time was 17-18 degrees, i.e. approximately two-fifths of the total loss in gravity (c.f. the corresponding loss in the pipe which was approximately one-third of the total loss). " Soluble nitrogen " reached its minimum in eight days as in pipe F. This minimum (1·4 mg.N/100 ml., i.e. the average of analyses on four consecutive centrifuged* samples—not the value on the 8th day) is 19% (approximately) of the initial value 7·5 mg.N/100 ml. (c.f. 1·5 mg.N/100 ml., and approximately 19%, the corresponding data for minimum " soluble nitrogen " of the fermenting juice in the pipe). Rather more than 80% of the " soluble nitrogen " was therefore readily available to the yeasts, as in the fermentation in the pipe. The loss in specific gravity associated with this utilisation of available " soluble nitrogen " was 17-18 degrees (i.e. about two-fifths of the total fall in gravity). Fermentation continued at a slightly diminished rate after the available " soluble nitrogen " had been utilised. The remaining decrease in gravity up to the 25th day, took place without any change in the " soluble nitrogen " content of the fermenting juice. There was an *increase* of 50% in the " soluble nitrogen " during the period twenty five to seventy four days (c.f. the increase in pipe F) and this increase (1·4—2·1 mg.N/100 ml.) indicated an appreciable extent of autolysis.

" Nitrogen in suspension " is regarded as representative of yeast in suspension as explained in Part I of the Results and Discussion. Yeast in suspension in the fermenting juice (*Fig.* 2b) was initially greater than in the pipe and reached its maximum (1·7 mg.N/100 ml., as in the pipe) rather more quickly—four days as compared with five days for the pipe. The decrease in the amount of yeast in suspension from its peak value was appreciably more rapid than in the pipe and yeast in suspension had fallen to half its peak value by the 10th day. The significance of the *initial* decrease in the " nitrogen in suspension " cannot be accurately assessed (c.f. fermentation in the pipe).

The build up of " nitrogen in the lees " (yeast), *Fig.* 2b, closely paralleled that in the pipe. Also, the course and rate of growth of yeast in the two fermentations were very similar and did not differ appreciably in any way. Yeast growth during the fermentation, as determined by the *difference* between the *initial* " soluble nitrogen " and the *minimum* " soluble nitrogen," was substantially the same in the jar as in the pipe (i.e. per 100 ml. of fermenting juice) and was practically complete in eight days (as in the pipe).

### B. The Fermentation in the Glass Jar at 25°C.

This jar of juice was placed in the incubator at 25° C. (77° F.). Since this jar and the jar at outdoor temperatures were filled from the same small bulk of juice, strict comparison between the two fermentations is possible.

The juice at 25° C. fermented much more rapidly (Table III and *Fig.* 3a) than that at the lower temperature. The " lag " period (corresponding to the " lag " phase of yeast-growth) was of approximately equal length in the two fermentations. The time taken from the end of the " lag " period for a decrease

* The *filtered* sample is not considered in this calculation.

in specific gravity of 38 degrees was six and a half days (c.f. twenty-four days at the lower temperature), a rate of almost 6 degrees per day, i.e. the rate of fermentation was *four times* as great as at the lower temperature. During the period one to four days the fall in specific gravity was at the rate of 9 degrees per day. In ten days (S.G. $1003\frac{1}{2}$) the fermentation was almost complete and the lowest gravity recorded was $1001\frac{1}{2}$, i.e. a total loss of 45 degrees of specific gravity.

Nine-tenths of the loss in " total nitrogen " which occurred during the whole of the fermentation, took place during the first four days and the loss in gravity during this time was 30 degrees, i.e. approximately two-thirds of the total loss in gravity (c.f. the corresponding loss at the lower temperature which was approximately two-fifths of the total loss in gravity). " Soluble nitrogen " reached its minimum (1·5 mg.N/100 ml.) in *three* days (c.f. eight days at the lower temperature). The minimum " soluble nitrogen " was 20% of the initial " soluble nitrogen " and this means that 80% of the " soluble nitrogen " was in a form easily available for yeast growth, as at the lower temperature. The loss in specific gravity associated with this utilisation of available " soluble nitrogen " was 30 degrees, i.e. approximately two-thirds of the total fall in gravity (c.f. two-fifths of the total fall in gravity at the lower temperature).

There was no change in the rate of fermentation until some time after all the available " soluble nitrogen " had been utilised. During the period three to six days " soluble nitrogen " remained unchanged but it increased markedly from 1·5—1·9 mg.N/100 ml. during six to eight days. This increase in " soluble nitrogen," 0·4 mg.N/100 ml., indicated a smaller extent of autolysis than that which occurred in the cider at the lower temperature over a longer period of time. The period during which this autolysis at 25° C. occurred is of special interest. It took place *before* the " suspended nitrogen " had finished sedimenting out and, in fact, coincided with the last two days of sedimentation of the yeast (see next paragraph). Furthermore, the *amount* of " nitrogen in suspension " (" yeast ") which sedimented during these two days could account quantitatively for the increase observed in the " soluble nitrogen." It is possible, therefore, that it was the yeast which deposited during the last two days of sedimentation (six to eight days) which was autolysed, but there is no proof and the suggestion is brought forward only as a possible explanation of the experimental data. The results suggest that autolysis occurred between the 6th and 8th days of fermentation during which period the fermentation was still proceeding at a moderately rapid rate. These conclusions concerning autolysis are of particular interest but must be regarded as tentative. To ascertain, with reasonable certainty, the period during which autolysis did, in fact, occur, it would be necessary to determine and compare the " soluble nitrogen " contents of *several* samples of cider withdrawn at, or near, the start and during the period of autolysis.

" Nitrogen in suspension " (yeast) rapidly (two days) reached its peak, 3·0 mg.N/100 ml., which was much greater than that at the lower temperature (*Fig.* 3b). The total amount of yeast present in each of the two fermentations at the times when " yeast in suspension " was at its maximum was approximately the same. It follows therefore that a much greater proportion of the yeast which had grown was in suspension (when at its peak) in the fermentation at the higher temperature. The yeast in suspension decreased fairly rapidly from its peak value and was equivalent to 0·6 mg.N/100 ml. on the 6th day. During this period 25 degrees of specific gravity were lost. At the lower temperature, yeast in suspension was equivalent to 0·6 mg.N/100 ml. after ten

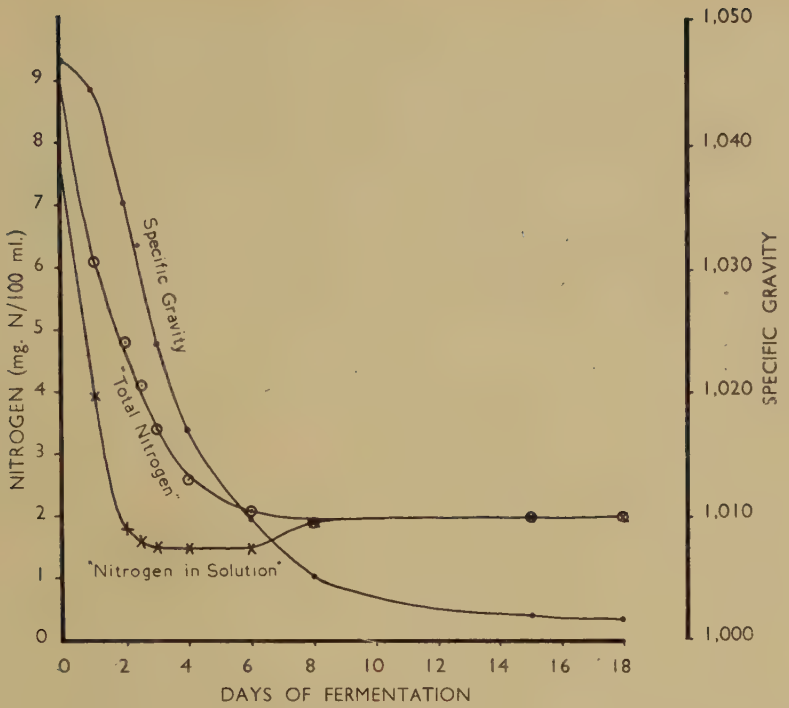


*Fig.* 3a. Fermentation in the Glass Jar at 25° C. Specific Gravity Curve and Changes in "Total Nitrogen" and in "Nitrogen in Solution" of the Fermenting Juice.

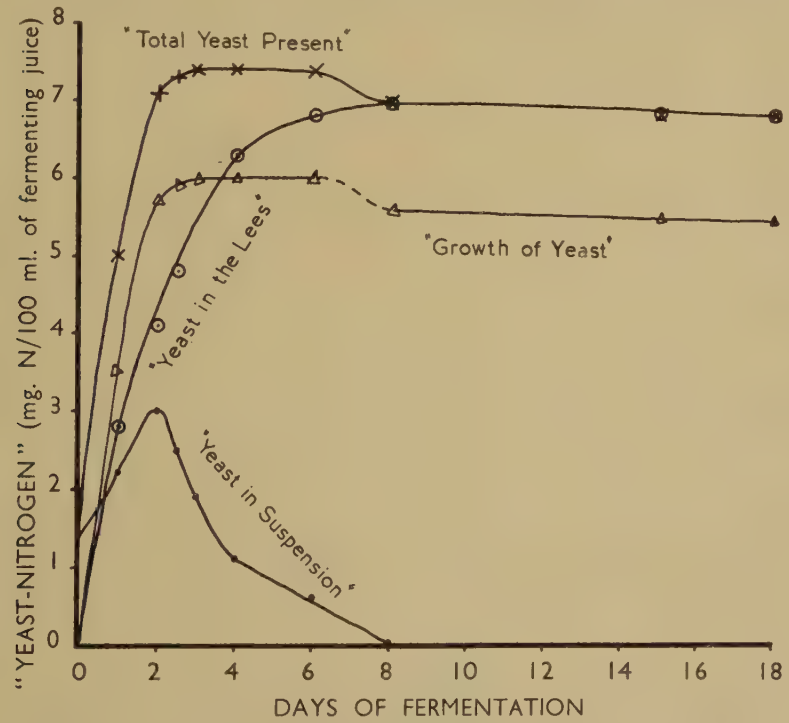
"YEAST-NITROGEN" (mg. N/100 ml. of fermenting juice)
"Total Yeast Present"
"Growth of Yeast"
"Yeast in the Lees"
"Yeast in Suspension"
0 1 2 3 4 5 6 7 8
0 2 4 6 8 10 12 14 16 18
DAYS OF FERMENTATION

*Fig.* 3b. Fermentation in the Glass Jar at 25° C, showing the Growth and Distribution of "Yeast" in the Fermenting Juice.

N.B. 1. The time-scale in the above graphs is larger than in *Figs.* 1a, 1b and 2a, 2b.
2. The ordinates in *Fig.* 3b represent "yeast-nitrogen." These values are taken as representative of yeast (see text).

## TABLE III.

FERMENTATION IN THE GLASS JAR AT 25° C.

CHANGES in the NITROGEN CONTENT of the Fermenting Juice. Table also gives Values (Calculated from the Nitrogen-Data) Representative of the GROWTH and DISTRIBUTION of YEAST in the Fermenting Juice. (All nitrogen contents are expressed as mg.N/100 ml. fermenting juice or cider).

| Days of Fermentation | Specific Gravity | NITROGEN in the Fermenting Juice | | "YEAST-NITROGEN" | | | |
|---|---|---|---|---|---|---|---|
| | | "Total Nitrogen" | "Nitrogen in Solution" | "*Total Nitrogen*" minus "*Soluble Nitrogen*" i.e. "*Nitrogen in Suspension*" Representative of *Yeast in Suspension* | *Decrease in* "*Total Nitrogen*" Representative of "nitrogen in the lees" and of *Yeast in the Lees* | *Decrease in* "*Soluble Nitrogen*" Representative of *Growth of Yeast* | "*Initial Total Nitrogen*" minus "*Soluble Nitrogen*" Representative of *Total Yeast Present** |
| 0 | 1,046½ | 8·9 | 7·5 | 1·4† | 0·0 | 0·0 | 1·4† |
| 1 | 1,044 | 6·1 | 3·9 | 2·2 | 2·8 | 3·6 | 5·0 |
| 2 | 1,035 | 4·8 | 1·8 | 3·0 | 4·1 | 5·7 | 7·1 |
| 2½ | 1,032 | 4·1 | 1·6 | 2·5 | 4·8 | 5·9 | 7·3 |
| 3 | 1,023½ | 3·4 | 1·5‡‡ | 1·9 | 5·5 | 6·0 | 7·4 |
| 4 | 1,016½ | 2·6 | 1·5‡‡ | 1·1 | 6·3 | 6·0 | 7·4 |
| 6 | 1,010 | 2·1 | 1·5‡‡ | 0·6 | 6·8 | 6·0 | 7·4 |
| 8 | 1,005 | 1·9 | 1·9 | 0·0 | 7·0 | 5·6 | 7·0 |
| 15 | 1,002 | 2·0 | 2·0 | 0·0 | 6·9 | 5·5 | 6·9 |
| 18 | 1,001½ | 2·0 | 2·0 | 0·0 | 6·9 | 5·5 | 6·9 |

‡‡ Value to which the "soluble nitrogen" falls after utilisation by the yeasts of the readily available soluble nitrogenous compounds of the juice.

* "*Total Yeast Present*" at any time is that present at the start of the experiment (proportional to 1·4 mg.N/100 ml.) plus the yeast which has grown (see preceding column) up to that time.

† This value, 1·4 mg.N/100 ml., is representative of the amount of yeast present (in suspension) in the juice at the start of the experiment.

days of fermentation, but the loss in gravity was only 21 degrees. *The more rapid rate of fermentation at the higher temperature is therefore associated with a greater amount of yeast in suspension.* Yeast in suspension, as indicated by the nitrogen data, fell to zero by the 8th day when the gravity was 1005. There did not appear to be any appreciable amount of yeast in suspension during the remainder of the fermentation (S.G. 1005 to 1001½).

"Nitrogen in the lees" (yeast) increased progressively during the first eight days of fermentation and then decreased only very slightly. Growth of yeast was practically complete in three days, as compared with eight days

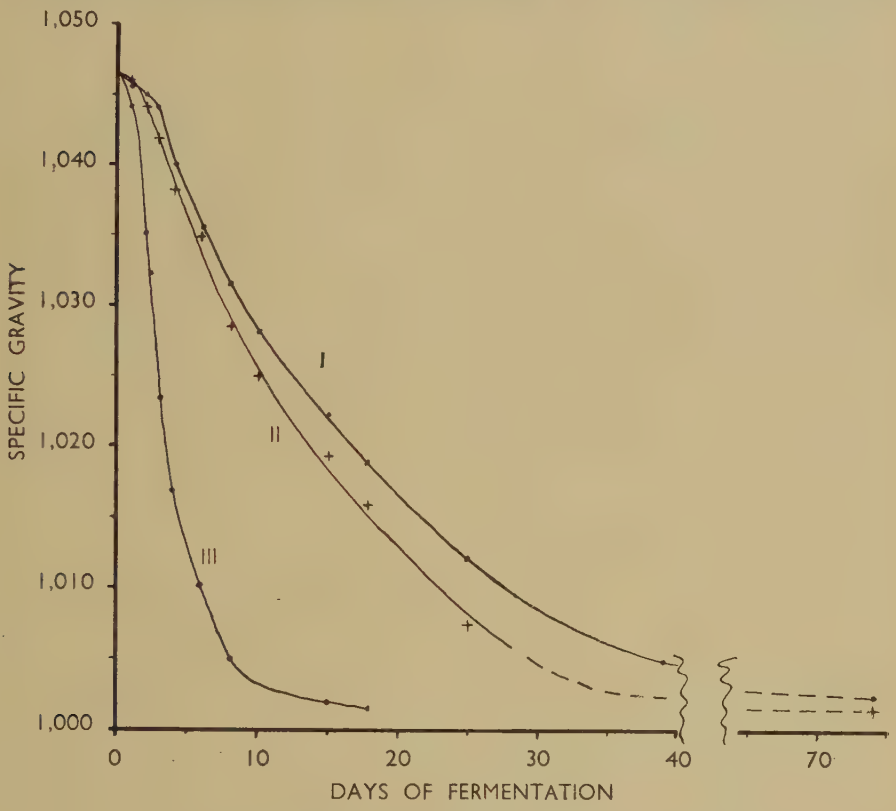


*Fig.* 4. Specific Gravity Changes During the Three Fermentations.

Curve I. Fermentation in Pipe F at Outdoor Temperatures.
„ II. Fermentation in the Glass Jar at Outdoor Temperatures.
„ III. Fermentation in the Glass Jar at 25° C.

at the lower temperature. The increase in "soluble nitrogen" which occurred as the result of autolysis is responsible for the apparent decrease in growth of yeast (*Fig.* 3b) as explained in Part I of the Results and Discussion. The different values for "total yeast *present*" on the 6th and 8th days may be similarly explained.

## GENERAL DISCUSSION.

This study of the nitrogen changes which occur during the fermentation of an apple juice under different conditions has yielded observations and conclusions which give some real insight into the manner in which a fermentation proceeds.

The nitrogen changes and the associated growth and distribution of the yeast in the fermenting juices have been described in detail for each of the three fermentations and only the more important aspects of the work can be briefly discussed here.

The fermentations in the pipe and in the glass jar at outdoor temperatures were very similar and no fundamental difference was observed. Further comparative studies of a similar nature are required, however, before it can be stated that the small-scale fermentation is truly representative of fermentation on the larger scale. In view of the similarity of the two fermentations at outdoor temperatures the following comments may in general be regarded as applicable to either; the fermentation at 25° C. will then be discussed separately.

The manner in which the utilisation of the available soluble nitrogenous compounds proceeded presents many features of interest; the rapidity with which these compounds were utilised; the proportion (approximately 50%) which was utilised during the "lag" period of fermentation; the almost constant rate at which the utilisation proceeded—the rate decreasing markedly only when utilisation was almost complete; the relatively small decrease in specific gravity associated with the utilisation of all, or most, of the readily available soluble nitrogen. The proportion of the "soluble nitrogen" which was *readily available* to the yeasts was approximately 80%. Consideration of the data for "soluble nitrogen" (Table II) suggests that a *further fraction* of the soluble nitrogenous compounds was utilised by the yeasts after the readily available soluble nitrogen had been utilised. More experimental evidence is required before this conclusion can be established, since the differences between the nitrogen contents concerned are small and are of the same order of size as the experimental errors of the nitrogen determinations at these low concentrations of nitrogen.

The above observations on utilisation of "soluble nitrogen" are indicative of the course of growth of the yeast. Yeast growth was rapid and proceeded at an almost constant rate until growth was practically complete; yeast growth had proceeded half-way to completion before there was any appreciable decrease in specific gravity in the fermenting juice and the decrease in specific gravity was still only relatively small when yeast growth was almost complete.

The accumulation of yeast in the lees was very rapid. At *a very early stage* in the fermentation (five days) lees-yeast had reached more than half its maximum and it was near the maximum after only ten days, i.e. at an early stage of the fermentation. It appears, therefore, that most of the yeast which grew during the fermentation sedimented very rapidly into the lees.

The relative amounts of yeast which were in suspension at different stages in the fermentation are of particular interest; yeast in suspension increased rapidly and had reached three quarters of its peak value almost before the end of the "lag" period of fermentation (three days)—an unexpected finding. Fermentation then became vigorous, the yeast in suspension rapidly reaching its peak value. The amount of yeast in suspension *then progressively decreased although the rate of fermentation did not alter greatly.* When yeast in suspension had fallen to one third of its peak value, fermentation was still vigorous although less so than when yeast in suspension was *at* its peak value. Quite different amounts of yeast in suspension were therefore associated with vigorous fermentation.

Yeast in suspension, even when at its peak, was only a small fraction of the total yeast present.

As already stated, knowledge of the relative amounts of *viable* and *dead* yeast cells which were present in the fermenting juices is not available.

The experimental data relating to autolysis has already been discussed reasonably fully in the appropriate section. No comparison of the relative extents of autolysis which occurred in the fermenting juice in the pipe and in the glass jar has been made, because of the difficulty of assessing these degrees of autolysis in fermenting vessels which differ so greatly in size and shape as the pipe and the jar (see also the introductory paragraphs of Part II of the Results and Discussion).

The fermentation of the juice at 25° C. proceeded much more rapidly than that at the lower temperature. Growth of yeast was much more rapid in the juice at the higher temperature as indicated by the more rapid utilisation of the soluble nitrogenous compounds. The amount of yeast growth, however, which occurred during each of the two fermentations was approximately the same.

The extent of the autolysis of yeast which occurred in the fermenting juice at the higher temperature was less than at the lower temperature although over a shorter period of time. This autolysis apparently occurred at an early stage, while the fermentation was still vigorous, but further experimental evidence is required before the period during which the autolysis took place can be ascertained with any degree of certainty.

The ciders prepared during the experimental work described in this paper were free from acetification and were typical Bramley ciders. The flavour and aroma of the cider which had stood on its lees for three months was similar to that of the corresponding cider which had been removed from the lees at the appropriate time and no change in flavour due to autolytic products could be detected. The high acidity of these ciders, however, tends to mask slight differences in flavour.

The detailed manner in which *each* of these fermentations has been studied was necessary so that effective comparison of fermentation under the different conditions chosen could be adequately made. The comparison of the fermentations at different temperatures is of obvious practical interest ; although less obvious, this is true also of the comparison between fermentation on the small scale and on the larger scale. Concerning the more fundamental aspect of this work, many observations have been made which show the need for further investigation (e.g. of autolysis)—its value will become more apparent in subsequent research. It is obvious, for example, that the *types* of nitrogen compounds in apple juice will have to be examined in relation to their availability to the different types (or varieties) of yeast which are present in the juice. Elements other than nitrogen require investigation in relation to fermentation of apple juice—in particular *phosphorus*, which is known to be of fundamental importance in fermentation processes.

The knowledge gained from this work, of the nitrogen changes and of the growth and distribution of yeast in the fermenting juice is applied, as described in the following paper, to the study of the mechanism of the method of controlling fermentation by centrifuging.

## SUMMARY.

(1) The fermentation of a Bramley juice has been studied with particular reference to the changes in the nitrogen content of the fermenting juice and the associated changes in specific gravity.

(2) " Total nitrogen " and " soluble nitrogen " in the fermenting juice were estimated at different stages of the fermentation.

(3) The course of the utilisation by the yeasts of the " soluble nitrogen " of the fermenting juice has been studied and the fraction of the soluble nitrogen which was easily available to the yeasts has been determined. There may be a further fraction of the soluble nitrogen which is less readily available to the yeasts.

(4) Estimates of the relative amounts of yeast present at different stages in the fermentation have been obtained by calculation from the nitrogen-data. From these estimates, the *growth* of yeast during the fermentation (total yeast formed) and its *distribution* in the fermenting juice (yeast in suspension—yeast in the lees) have been characterized.

(5) Observations on the autolysis which occurred during (or after) fermentation have been made.

(6) The solid material in a sample of lees has been examined and its yeast content estimated.

(7) The fermentation of the juice has been studied under different conditions : (1) fermentation in a pipe (cask) at outdoor temperatures ; (2) fermentation in a glass jar at outdoor temperatures ; (3) fermentation in a glass jar at 25° C.

(8) Comparisons have been made between : (a) fermentation in the pipe and that in the glass jar ; (b) fermentation in the glass jar at 25° C. and that in the glass jar at outdoor temperatures.

(9) No important difference was observed between the fermentation of a small volume of juice in a glass jar and fermentation of a relatively large volume of juice in a wooden pipe.

(10) The knowledge gained of the distribution of yeast in a fermenting juice at different times during the fermentation is of *practical* value, inasmuch as it contributes to the understanding of the processes of racking, centrifuging, and filtration which are used so extensively in cider-making.

## ACKNOWLEDGMENTS.

Thanks are due to Messrs. R. J. Hathway and J. H. Llewellyn for assistance in the cider-house and to Miss Yvonne May and Mr. D. Slater for help with the analytical work.

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# THE RÔLE OF NITROGEN IN FERMENTATION. II. OBSERVATIONS ON NITROGEN IN RELATION TO THE MECHANISM OF THE "FERMENTATION CONTROL OF CIDERS BY THE CENTRIFUGE METHOD."

By L. F. BURROUGHS and S. W. CHALLINOR.

Methods of controlling the fermentation of apple juice are of great importance to the cider maker. Such control may be effected by restricting the action of the yeasts by chemical means (addition of sulphur dioxide) or by mechanically removing the yeasts by physical methods such as racking, centrifuging or filtering. The advantages and disadvantages of these methods are discussed by Charley (1) and (6).

The use of the centrifuge for fermentation control in cider-making has been extensively studied (1-6) from the point of view of clarification of the cider and also as a means of retaining natural sweetness in cider. Barker and Grove (2) who first introduced (1930) the centrifuge method of control of fermentation have shown that, apart from the advantage of the saving of sugar, naturally sweet ciders obtained by the centrifuge method are markedly superior in quality to the artificially sweetened product.

It is of obvious importance that the principles of this valuable method of control of fermentation should be completely understood so that the centrifuge may be employed to best advantage. Charley (1, 4, 5 and 6) has contributed largely to our knowledge of the principles involved. He has shown (a) that the specific gravity of the fermenting juice must be allowed to fall appreciably (e.g. 10-12 degrees from the original gravity) before centrifuging, (b) that a single centrifuging may be effective in checking the fermentation of the juice, (c) that the method is more effective on low-nitrogen juices than on juices with a high nitrogen content, (d) that the addition of sulphur dioxide after centrifuging materially aided the efficiency of the centrifuge treatment.

In a study of the effect of centrifuging on the yeasts present in the fermenting juice Charley (4) has shown that the centrifuge brings about a specific removal of the larger types of yeast. Charley (5) has also studied the relationship between changes in nitrogen content in juices and ciders and the subsequent rates of fermentation of the centrifuged ciders.

As a result of these investigations, Charley has concluded that the principle of this method of control of fermentation depends not upon the removal of yeast as such but rather upon the removal of *nitrogen* in the form of yeast, and that the removal of mineral material such as phosphates might also be concerned. He was, however, unable to show a definite relationship between the relative amounts of nitrogen removed by *centrifuging* a fermenting juice at different specific gravities and the efficiency of the process in checking fermentation (5). This aspect of the problem is further examined in the present paper.

A somewhat different approach to the problem from that described above has been adopted. The Bramley juice, the fermentation of which

(in pipe F) has been carefully studied as described in the preceding paper (7), was chosen for the work, so that the nitrogen changes which took place in the fermentations which had been checked by centrifuging could be compared with those which occurred during the natural, i.e. unchecked, fermentation.

## EXPERIMENTAL.

The pressing of the juice from the Bramley apples, the bulking of this juice and its distribution into pipes has been described in the preceding paper (7). Six pipes (labelled B, G, A, C, D and F) of the juice, original specific gravity 1046½, were used in this experiment. The pipes were situated in the cider-house yard and the juice allowed to ferment (12.11.48). Fermentation in four of these pipes was checked by centrifuging after varying falls in the specific gravity of the juice had occurred. Thus, pipe B was centrifuged at a gravity of 1024, pipe G at 1018, pipe A at 1012 and pipe D at 1008 (Table I). The juice in the two pipes D and F was allowed to ferment to dryness (S.G. 1002½) before being centrifuged. The centrifuged ciders were stored in the cider house.

The procedure adopted for each pipe was as follows : Immediately before centrifuging, a sample of the fermenting juice was removed from the middle of the pipe (without causing any disturbance of the lees) for specific gravity and nitrogen determinations. The juice (or cider) was then centrifuged through the Sharples Supercentrifuge (the lees were not centrifuged) and collected in a metal Prodorglas-lined tank. A sample was then taken from the bulked centrifuged cider (for specific gravity and nitrogen determinations) which was then pumped into a hogshead and stored in the cider-house. Further samples were withdrawn from each hogshead during the storage period (11.3.49) for specific gravity determinations only and another sampling was made at the end of the storage period (20.4.49) for nitrogen and specific gravity determinations. The period of storage varied from 85 to 142 days according to the time of centrifuging (Table I).

" Total nitrogen " and " soluble nitrogen " in these samples was determined by the Kjeldahl method. Interpretation of this data and its conversion into data representative of yeast was carried out as previously described (7) and only brief comment is necessary here. Thus (i) samples did not include material from the lees, (ii) " total nitrogen " was determined on the uncentrifuged sample and includes both " nitrogen in solution " and " nitrogen in suspension " (the latter is regarded as representative of yeast in suspension), (iii) " nitrogen in solution," i.e. the nitrogen of the soluble nitrogenous compounds of the juice or cider, was determined on the centrifuged cider or on the centrifuged sample.

It is necessary to explain why a distinction is made between centrifuged cider and centrifuged sample. In the experiments described in the preceding paper (7), bulk-centrifuging of the ciders was not a special feature of the experiment : each *sample* of fermenting juice (or cider) was (a) analysed directly without centrifuging to determine " total nitrogen," i.e. " nitrogen in suspension " plus " nitrogen in solution," and (b) analysed after centrifuging on the laboratory centrifuge to determine " nitrogen in solution." In the work now under consideration the ciders themselves were centrifuged *in bulk* at particular specific gravities, as already stated, and the nitrogen content of these centrifuged ciders was then regarded as consisting of " soluble nitrogen " only, i.e. these samples themselves were not further centrifuged. The Sharples-centrifuged cider was clear and of a degree of clarity comparable with that of a

## TABLE I.

### Fermentation Control by the Centrifuge Method.

*Specific Gravity and Nitrogen Content of the Centrifuged Ciders.*

The initial nitrogen content of the juice was : "Total Nitrogen" 8·8 mg. N/100 ml. of juice ; "Soluble Nitrogen" 7·7 mg. N/100 ml. of juice—see Ref. (7). The juice had an original gravity of 1046½.

| Pipe | Period of Fermentation (Days) | Specific Gravity at time of Centrifuging * | Decrease in Specific Gravity before Centrifuging (Degrees) | Duration of Storage Period (Days) ⊛ | Specific Gravity during Storage Period | | Loss of Specific Gravity during Storage Period (Degrees) | Nitrogen Content of Cider (mg. N/100 ml.) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 11/3/49 | 20/4/49 (End of Period) | | At time of Centrifuging (i.e. *Beginning* of Storage Period) | | At *End* of Storage Period (20/4/49) | |
| | | | | | | | | "Total Nitrogen" | "Nitrogen in Solution" † | "Total Nitrogen" | "Nitrogen in Solution" ‡ |
| B | 17 | 1,024 (29/11/48) | 22½ | 142 | 1,016 | 1,014 | 10 | 2·3 | 1·5 | 1·3 | 1·3 |
| G | 19 | 1,018 (1/12/48) | 28½ | 140 | 1,014 | 1,013½ | 4½ | 2·1 | 1·6 | 1·2 | 1·1 |
| A | 20 | 1,012 (2/12/48) | 34½ | 139 | 1,011 | 1,011 | 1 | 2·4 | 1·6 | 1·2 | 1·1 |
| C | 31 | 1,008 (13/12/48) | 38½ | 128 | 1,006½ | 1,006 | 2 | 2·3 | 1·7 | 1·2 | 1·1 |
| D | 74 | 1,002½ (25/1/49) | 44 | 85 | 1,002½ | 1,002½ | 0 | 1·8 | 1·9 | 1·6 | 1·5 |
| F | 74 | 1,002½ (25/1/49) | 44 | 85 | 1,002½ | 1,002½ | 0 | 1·8 | 1·8 | 1·5 | 1·4 |

* Through a Sharples Supercentrifuge. Time of centrifuging is the beginning of the Storage Period.

⊛ Duration of Storage Period varied according to time of centrifuging.

† Samples were taken from the bulked centrifuged cider (Sharples) and analysed without further centrifuging (see column 3 for S.G. data and date of centrifuging).

‡ Samples were taken direct from the stored cider and centrifuged on the laboratory centrifuge (2,500 r.p.m. for 30 mins.) before analysis.

sample of cider which had been centrifuged on the laboratory centrifuge. Furthermore, the "soluble nitrogen" contents of the ciders from pipes B, G and A after passing through the Sharples-centrifuge (Table I) were comparable with the minimum "soluble nitrogen" (1·5 mg.N/100 ml.) of the

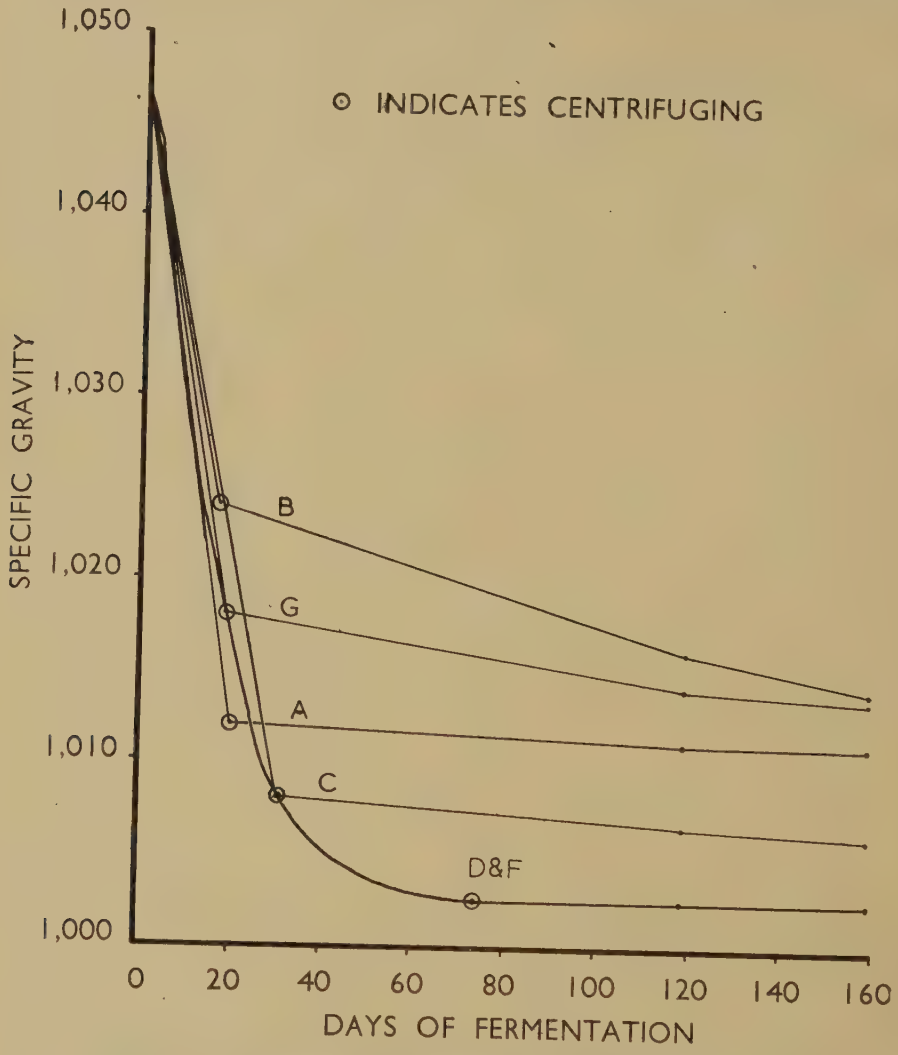


*Fig.* 1. Specific Gravity Changes in a Fermenting Juice, the Fermentation of which has been checked by Centrifuging at different Specific Gravities.

cider (laboratory-centrifuged samples) in pipe F (7). The two centrifuge treatments may therefore be regarded as of approximately equal efficiency in removing suspended nitrogenous material from the juice or cider.

## RESULTS AND DISCUSSION.

Fermentation of the juice in pipe F and the associated nitrogen changes which occurred during the fermentation have previously been fully described (7); the results of this work need not be summarised here but frequent reference to this paper (7) will be necessary. The fermentation in pipe F

was not checked and serves as a control for the fermentations which were checked by centrifuging as described in this paper. The fermentation in pipe D was also allowed to proceed without checking and may be regarded as another control.

**Specific Gravity Changes.**

The fermentation of the juice in pipe F was rather slower than expected for the variety (Bramley). The fermentation curve is shown on *Fig.* 1 but is more clearly seen on *Fig.* 1a which is reproduced from the preceding paper

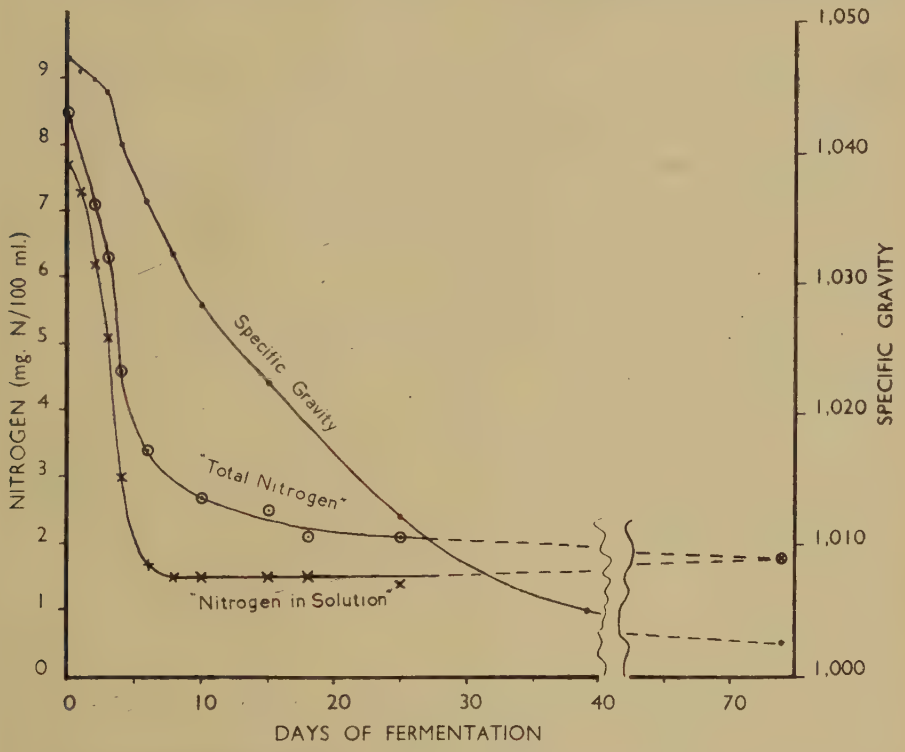


*Fig.* 1a. Fermentation in Pipe F at Outdoor Temperatures. Specific Gravity Curve and Changes in "Total Nitrogen" and in "Nitrogen in Solution" of the Fermenting Juice.

(7). *Fig.* 1a also shows the changes in "total nitrogen" and in "soluble nitrogen" which occurred during this control-fermentation.

The fermentations which were checked by centrifuging will now be considered (Table I and *Fig.* 1). The decreases in specific gravity prior to centrifuging, which occurred in the juice in pipes B, G, A and C were $22\frac{1}{2}$, $28\frac{1}{2}$, $34\frac{1}{2}$ and $38\frac{1}{2}$ degrees respectively. During the *storage period*, which varied according to time of centrifuging (142-128 days for these four ciders) there was very little further decrease in gravity except in the ciders B and G where the loss in gravity was 10 and $4\frac{1}{2}$ degrees respectively. The loss in gravity in the cider from pipe B occurred during 142 days of storage i.e. an average loss of 2 degrees of gravity per four weeks ; the average loss in gravity of the cider from pipe G was only 1 degree per four weeks. It will be seen that the check to the fermentation of the ciders from pipes G, A and C was extremely effective. The fermentation of the cider from pipe B was not

checked so efficiently by the centrifuge treatment, although from the practical point of view the degree of control of the fermentation of this cider would be considered satisfactory for most purposes. Comparison of the specific gravity changes which occurred in these four ciders after centrifuging, with the specific gravity changes in cider F (and also D), which was not centrifuged until fermentation was complete, shows that the check to the fermentation of each of these four ciders was very effective.

**Changes in the Nitrogen Content of the Centrifuged Ciders.**

(a) *In Relation to Centrifuging.* The " total nitrogen " contents of ciders B, G, A and C at the time of centrifuging (i.e. the nitrogen content of samples withdrawn from the ciders immediately before centrifuging) did not differ materially from each other (Table I). After the centrifuging process, which removed yeast in suspension (" nitrogen in suspension ") the nitrogen contents of the ciders, i.e. the " soluble nitrogen " contents, were also approximately the same. There was, therefore, no significant difference in the amounts of " nitrogen in suspension " in these ciders. Accordingly, there was *no significant difference in the amounts of " nitrogen " removed from the ciders by the respective centrifuge treatments.*

As expected, the ciders D and F which had been allowed to ferment to dryness (S.G. $1002\frac{1}{2}$) before centrifuging, contained no appreciable amount of yeast in suspension as shown by the nitrogen content of these ciders before and after centrifuging.

(b) *During Storage.* The " soluble nitrogen " content of each of the six ciders (Table I) at the beginning of the storage period was rather greater than the corresponding value at the end of the period. There does not appear to be any direct relationship between the decreases in the " soluble nitrogen " contents of these ciders which occurred during the storage period and the losses in specific gravity which took place in these ciders during this period.

**The Mechanism of the Method of Control of Fermentation.**

In the introductory paragraphs of this paper the work of Charley on this method of control of fermentation has been reviewed. The results of the experiments described in this paper will be considered here mainly in respect of the changes which occurred in the nitrogen contents of the fermenting juices *prior* to centrifuge treatment.

Experimental evidence has been brought forward to show (i) that the fermentation of each of the four ciders B, G, A and C was materially checked by a single centrifuging although differences in the degree of control of fermentation in the different ciders were observed, (ii) that the amounts of " nitrogen in suspension " (yeast in suspension) removed from each of these ciders by the centrifuge treatment were approximately the same. The degree of control of fermentation cannot therefore be directly related with the amount of " nitrogen in the form of yeast in suspension " which was removed by the centrifuge treatment. Such a relation, however, could not be expected since the yeast which grows during fermentation is removed from a centrifuged cider in two ways (i) by sedimentation into the lees *prior to centrifuging*, (ii) by the process of *centrifuging* which *removes only the yeast in suspension at the time of centrifuging.*

A relation might, however, be expected between the degree of control of fermentation and the total amount of " nitrogen " in the form of yeast which

is removed from the fermenting juice in these *two* ways. This possibility will now be examined.

The relative amounts of yeast (and therefore of " nitrogen ") which were present (a) in the lees and (b) in suspension at different times during the fermentation of this Bramley juice (in pipe F) have been ascertained in the work described in the preceding paper (7). It is not, however, necessary to determine these relative amounts *separately*. A more direct approach is possible by consideration of the changes in the " soluble nitrogen " content of this juice which occurred as the fermentation progressed and, in particular, of the amount of " *soluble nitrogen* " *which remained in the cider after centrifuging.*

These changes in the " soluble nitrogen " content of the fermenting juice in pipe F have been examined and described (7) and are illustrated in *Fig.* 1a which is reproduced here and to which reference has already been made. From the graph it is seen (i) that utilisation of the " soluble nitrogen " was rapid, the readily available " soluble nitrogen " (which constituted 80% of the *initial* " soluble nitrogen ") being utilised in only seven to eight days and (ii) that the associated decrease in specific gravity was only 15 degrees.

It follows that if the fermenting juice is centrifuged *before* all the readily available " soluble nitrogen " has been utilised (eight days) then some " nitrogen " readily available for yeast growth will remain and the check to fermentation could not be expected to be complete. On the other hand, if centrifuging is carried out *after* this utilisation is complete, then the centrifuged cider would contain little or no available " nitrogen " and growth of yeast would be prevented or considerably restricted, i.e. fermentation would be effectively checked.

If this hypothesis is correct, efficient control of fermentation would depend upon whether sufficient time had elapsed before centrifuging for complete utilisation of the readily available " soluble nitrogen " to take place. The data relating to the " soluble nitrogen " contents of the four ciders B, G, A and C (Table I) at the time of centrifuging will now be examined from this point of view.

The following observations are relevant :

(i) The " soluble nitrogen " contents of these four ciders at the time of centrifuging were (a) approximately equal and (b) did not differ appreciably from the " minimum soluble nitrogen " of the fermenting juice in pipe F (1·5 mg.N/100 ml.) i.e. the value to which the " soluble nitrogen " content of this juice decreased after complete utilisation of the readily available " soluble nitrogen " had taken place (7).

(ii) The time taken for complete utilisation of the readily available " soluble nitrogen " of the juice in pipe F was only seven to eight days (7). If this fact had been known when the centrifuging experiments were in progress, then the first centrifuging of the cider (that from pipe B) would have been carried out earlier, i.e. before the eighth day of fermentation. In spite of the fact that this knowledge was not available, the first centrifuging of cider had been planned to take place earlier than it was in fact carried out, but the centrifuging was delayed owing to practical difficulties. From the observations made above, (i) and (ii), on the " soluble nitrogen " content of these four ciders at the time of centrifuging it follows that the readily available " soluble nitrogen " of *each* of these ciders had already been utilised before centrifuging took place.

It would be expected, therefore, that the fermentation of each of these

four ciders B, G, A and C would have been effectively checked by the centrifuge treatment. Each of these fermentations was, in fact, effectively checked by centrifuging but the efficacy of the check to fermentation varied. Thus the cider from pipe B lost 10 degrees of gravity during its long storage period of 142 days (Table I) as compared with (a) the cider from pipe G which lost only $4\frac{1}{2}$ degrees in 140 days of storage and (b) the ciders from pipes A and C the fermentation of which was almost completely checked. Although, therefore, the fermentation of each of these ciders may be regarded as having been checked effectively from the practical point of view, it must be accepted that the degree of control of the fermentation varied.

It is difficult to assess the significance of these variations in the degree of control of fermentation but the following interpretation of the results is suggested :

That the degree of control of fermentation :

(i) of cider B differed appreciably from that of cider G and that the difference *may* be significant ;

(ii) of cider G did not differ significantly from that of cider A (or C) ;

(iii) of cider B differed appreciably from that of ciders A and C, and that the difference is almost certainly significant.

If the interpretation of the results as set out in the preceding paragraphs is accepted then the following conclusions may be provisionally drawn :

(1) That effective control of the fermentation of a juice (or cider) may be expected if the juice is centrifuged after utilisation of the readily available " soluble nitrogen " is complete.

(2) That the mechanism of this method of control cannot *entirely* be explained on this basis of utilisation of the readily available " soluble nitrogen " of the juice, i.e. some other factor must be concerned.

Further experimental evidence is required before these two conclusions can be definitely established. Thus, knowledge of the behaviour of a cider which had been centrifuged before complete utilisation of the readily available " soluble nitrogen " had taken place, would have helped in the interpretation of the mechanism of this method of control of fermentation. It is known, however, from the work of Charley (see introduction), that effective control of fermentation is not obtained unless centrifuging is delayed until an appreciable fall in the specific gravity of the fermenting juice has taken place. It is suggested here that this delay in centrifuging is in fact necessary in order that utilisation of the readily available " soluble nitrogen " of the fermenting juice may occur. Experimental evidence in support of this suggestion is not complete, but it has been shown (7) that the time required for utilisation of the readily available " soluble nitrogen " of this cider in pipe F was seven to eight days and that the associated fall in specific gravity was 15 degrees. Since, however, no one of these ciders *was* centrifuged *before* complete utilisation of the readily available " soluble nitrogen " had taken place, it cannot be stated with *certainty* that the fermentation of the cider centrifuged at this early stage would *not* be checked.

The nature of the other factor which may be concerned in the mechanism of this method of control of fermentation is unknown. In the preceding paper (7) evidence is brought forward which suggests that, in addition to the readily available " soluble nitrogen," there is a second fraction of the " soluble nitrogen " of the juice which is less readily utilised by the yeasts. This fraction

of the "soluble nitrogen" may be concerned in the second factor. It is, however, possible that some other element such as phosphorus is concerned (5, 7). This possibility will be investigated in subsequent work in which the changes in nitrogen *and* in phosphorus which occur as the fermentation progresses will be followed.

## SUMMARY.

The fermentation of a Bramley juice has been checked at different specific gravities by the centrifuge method. Changes in the nitrogen content of the fermenting juice have been studied.

A further contribution to our knowledge of the mechanism of this method of control of fermentation has been made.

## ACKNOWLEDGMENTS.

Thanks are due to Messrs. R. J. Hathway and J. H. Llewellyn for assistance in the cider-house and to Miss Yvonne May and Mr. D. Slater for help with the analytical work.

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# NOTES ON CIDER MAKING IN SWITZERLAND AND FRANCE.

By S. W. CHALLINOR.

In the years before the war, parties of cider-makers from this country have made tours abroad to France, Switzerland and Germany to learn something of the methods of cider-making employed in these countries. A report on the tours made during the period 1925-37 has been written by Charley (1). It has now proved possible to resume these tours and in the early summer of 1948 a visit was made to Switzerland (previously visited in 1931), where seven factories, situated mainly in the area around Zurich, were visited. The writer travelled as one of the party and, when the official tour had concluded, visited the Research Station at Wädenswil. Problems concerned with cider-making in Switzerland were discussed with Dr. H. Luethi and his colleagues at Wädenswil. On the return journey a short time was spent in France and visits were made to cider and fruit juice factories and also to the Cider Research Station at Rennes. Some of the problems of cider-making in France were discussed with Professor J. Tavernier and his colleagues at Rennes.

At the time of the visits only certain sections of the factories were in operation but the general layout and plant were inspected and the processes involved in the manufacture of cider and of unfermented apple juice were discussed.

It is worthy of note that most of the factories visited produced both cider and unfermented apple juice. The production of this latter product is described by Dr. Pollard in an accompanying article (2).

## SWITZERLAND

### The Fruit.

The bulk of the apples used in Switzerland for cider-making could not be described as typical cider fruit in the sense in which the term is used in this country and in France. The tannin content of the fruit, as judged by the ciders which were tasted, was lower than that of the fruit used for cider-making in this country and in France. The apples used are of high acidity. Large quantities of pears are available in Switzerland and the pears are mixed with apples in order to reduce the over-all acidity. The proportion of pears in the mixed fruit is believed to be quite large. Fruit usually arrives by rail and is transferred into large bins (or silos) made of concrete with a bituminous lining. From the bins the fruit is carried to the mill by roller (aluminium alloy) conveyor. It was stated that the fruit used was in good condition and at a suitable stage of maturity. On the way to the mill the fruit is very thoroughly washed, e.g. by jets of water at high pressure. Efficient washing of the fruit is regarded as of considerable importance.

### Milling and Pressing.

The actual processes were not in operation at the time of the visits. Improved types of mill and press of recent design were in general use. Fruit is crushed by a worm and is then thrown between a phosphor-bronze rotor and serrated knives arranged around the inside of the drum in which

the rotor revolves. This type of mill is very efficient and is capable of pulping the fruit at the rate of 7-8 tons per hour.

The milled fruit is conveyed to the presses by pumping or by gravity according to the layout of the factory. These presses are of modern design and are capable of handling large quantities of crushed fruit. They are of the three-bay, turntable type and take three cheeses, each of $1\frac{1}{2}$ tons, per hour. Thus, the construction of the press is such that a cheese is built and is then rotated through 120° to receive its first pressing of 20 minutes duration. While this pressing is taking place a second cheese is built, in the position where the first cheese was formed, and the two cheeses are then rotated through 120°. A third cheese is then built up and during this time the first cheese receives its second pressing, and the second cheese its first pressing, and so the process goes on. The amount of pomace in each layer of a cheese is automatically regulated. Features of special interest are as follows: (1) each cheese is constructed of many thin layers (20-40) and this contributes to the very efficient juice extraction which is obtained: (2) the cheese is not broken up between pressings and this means (a) considerable saving of labour, (b) less oxidation of the juice still to be expressed; (3) the second pressing is carried out at a much higher pressure than the first. Pressures used are of the order of 100 lb./sq. in (on the racks) for the first pressing and much higher (two-three times greater) for the second pressing.

**Fermentation.**

It is noteworthy that fermentation by a selected yeast appeared to be the standard procedure in Switzerland. One of two wine-yeasts is used—either Fendant or Herrliberg—and the cultures are supplied by the Research Station at Wädenswil.

A full description of the fermentation procedure was not obtained but the salient features are as follows: the " starter " is prepared from the mother culture by inoculation into a small quantity (e.g. $\frac{1}{4}$—1 gallon) of the juice which has been sterilised by heat. After incubation until fermentation becomes active, this culture is used for the inoculation of a larger volume of sterilised juice and the process is repeated until a suitable volume of actively fermenting juice is obtained, e.g. 1—4% of the volume of juice to be fermented. Before this " starter " is added to the juice to be fermented it is desirable to treat the latter so that the activity of the wild yeasts and other micro-organisms present is restricted. This may be effected by the addition of a small quantity of sulphur dioxide to the juice. Centrifuging the juice in order to remove most of the micro-organisms present is another possible treatment. When sulphur dioxide is used the " starter " must not be added to the juice until after an interval of some hours. The added yeast is thus able to gain the upper hand so that a dominant fermentation is assured. Fermentation is carried out at a temperature of 20° C. (approx.) and is usually allowed to proceed until the specific gravity has fallen to 1,010 (approx.); the time taken is about three to four weeks or rather less if an unduly large proportion of pear juice is present. Fermentation at lower temperatures (e.g. below 10° C.) is possible with either of these selected yeasts; at one of the factories visited this fermentation is carried out in bitumen-lined concrete tanks.

A feature of interest was the use made of carbon dioxide produced during fermentation. The gas is piped from the fermentation tanks, washed three times and then passed through lime-wood charcoal into compressors. It is

used for pressure-storage of apple juice and, apart from the saving of cost, it was claimed that it has the advantage of imparting a better flavour to the juice than commercial carbon dioxide.

**Subsequent Processing.**

After fermentation, the cider is clarified by filtration through a plate filter or pulp filter of the usual type and then stored at a low temperature (1-2° C.) under a protective layer of carbon dioxide. Under these conditions undesirable secondary fermentations are inhibited. One such fermentation was mentioned in particular—the malolactic acid fermentation, which was said to be undesirable at this stage of the processing. At some of the factories visited the cider was conditioned at a higher temperature (12-14° C.) for two weeks before sale. This conditioning was stated to materially improve the flavour of the cider.

The addition of sugar to cider is not permitted in Switzerland and, consequently, the method of production of sweet cider is quite different from that used in this country. When sweet cider is required, unfermented apple juice or concentrate is added to the unsweetened cider. Sterilisation of the product is therefore essential and is effected by pasteurisation in bottle. This can be carried out at a lower temperature than for apple juice and, according to Dr. Luethi, cider is pasteurised at approximately 60° C. for 15-20 minutes (10 minutes " coming-up time," 15-20 minutes " holding time," and 20 minutes cooling by spray).

**The Ciders.**

Most of the ciders tasted were dry. Aroma and flavour were slightly vinous due to the wine-yeast used for fermentation but the characteristic apple flavour was lacking. All the ciders were brilliant and, compared with ciders made in this country, contained little tannin, much acid and were lightly carbonated. Swiss ciders differ greatly from English ciders and comparison is difficult. The ciders tasted were of a uniformly high quality. The most popular ciders were dry (about 1-3% natural sugar) and were believed to contain about 6-8% alcohol. Ciders sweetened with unfermented juice are apparently not as popular as dry cider. None of the ciders tasted contained detectable amounts of sulphur dioxide. Most of these ciders appeared to be free from any marked pasteurisation flavour.

Cider in an active state of fermentation (known as *Geraetz*) is also sold although none was available at the time of the visit.

**Other Products.**

Concentration of apple and pear juices at a low temperature (about 35° C.) was seen at two of the factories visited and the processes employed are described by Dr. Pollard (2). It was stated that some fermentation difficulties were encountered with de-acidified concentrates.

The extraction of pectin from apple pomace has received particular attention in Switzerland. Pomace-drying was carried out in all the factories visited and pectin extraction was in progress at one of the factories but little information concerning the method of extraction was given.

The production of vinegar from cider was in progress at two of the factories. Cider with a high alcohol content is used ; at one of the factories the cider was first concentrated, by freezing, to an alcohol content of 10-12%. The secondary fermentation, by which the alcohol is converted into acetic

acid, is accomplished by circulating the cider over corn cobs (Italian Process) or over wood shavings impregnated with acetic acid bacteria.

The production of cider-brandy was not seen but the distillation of fermented cherry pulp from steam-heated metal pots fitted with condensers was in progress at one of the factories visited.

**General.**

It may be of value to summarise some of the more important impressions of this tour : (1) The high standard of hygiene and cleanliness and the efficiency and standardisation of plant of modern design at most of the factories visited. (2) The general use of selected yeasts for fermentation with the attendant advantages of (a) greater degree of control of fermentation and (b) greater uniformity of product as compared with fermentation by the wild yeasts naturally present in the juice. (3) The extensive use of refrigeration (a) so that fermentation may be carried out at relatively low temperatures and (b) for the storage of cider under conditions which restrict the development of undesirable secondary fermentations. (4) The widespread use of bitumen-lined concrete tanks for storage of cider. (5) The use of unfermented apple juice instead of sugar for the sweetening of cider. (6) The production of both cider and unfermented apple juice at most of the factories visited. (7) The almost universal use of pasteurisation in order to produce a stable product.

## FRANCE.

The time spent in France was short and certain aspects only of the French methods of cider-making will be briefly discussed ; only two factories were visited.

**Pre-fermentation Processes.**

The nature of the French cider fruit of the bittersweet type and its suitability for cider-making are well known and require no more than mention here. The fruit is washed and then milled to a pulp in such a way that the pips are not disintegrated. After milling, the pulp is treated in one of two ways—it is either directly pressed or subjected to maceration.

*Maceration.* The milled fruit is allowed to stand in open tanks for 5-6 hours. During this process, extraction of pectin and of enzymes takes place and oxidation of the tannins also occurs. The oxidation is considered desirable for the following reasons : (i) it produces a deepening in the colour of the juice ; (ii) it assists in the subsequent clarification ; (iii) it is believed to improve the microbiological stability of the cider. It was stated that acetification did not occur to any appreciable extent during maceration. The milled fruit, whether macerated or not, is then pressed at a relatively low pressure and (at the two factories visited) the pomace subjected to the diffusion process.

*Defecation* (*keeving*). The expressed juice is then subjected to the process of defecation. This process is still extensively used in France and is believed to be advantageous in many respects. The juice is allowed to stand in open tanks at a low temperature (5-6° C.) for several days. Fermentation is restricted at this low temperature. During this time the natural pectic enzymes (pectases) of the juice become active and the enzyme activity may be assisted by the addition of a small quantity of a mixture of calcium carbonate and sodium chloride (*sel défécant*). A voluminous precipitate of pectic and nitrogenous substances is formed both at the top and bottom of the liquid.

The precipitation also brings about the removal of micro-organisms and in this way diminishes the risk of undesirable secondary fermentations. The chief advantage of the process, however, is the removal of nitrogenous compounds from the juice. According to Prof. J. Tavernier up to 40-50% of the total " nitrogen " of the juice may be removed in this way. Juices so treated have insufficient residual " nitrogen " for complete fermentation and the production of naturally sweet cider is greatly facilitated. It is essential that the fruit should be at a suitable stage of maturity (full maturity) if removal of nitrogen by this process is to be effective. When the process is unsatisfactory, fermentation may be checked by centrifuging or by filtration. Although defecation lasts several days it was stated that acetification troubles were not experienced. This may have been due in part to the use of sulphur dioxide for disinfection of the plant.

Maceration and defecation are only briefly described here. These processes have previously been described in detail by Charley (1).

**Fermentation.**

Various types of fermentation are practised :

(i) Fermentation in open tanks at ordinary temperature ; a low rate of fermentation is desired and a month may be taken for completion.

(ii) Partial fermentation to a gravity of 1025-1030 e.g. of defecated juices to give a naturally sweet cider ; if fermentation does not cease at the desired gravity it may be stopped by centrifuging or filtering followed by storage at 10° C. ; or the fermentation may be allowed to proceed unchecked and some of the dry cider thus produced is distilled to give a cider-brandy.

(iii) Fermentation under Pressure. (a) A type of Cidre Doux (sweet cider) is made by allowing a very slow fermentation to take place in closed vessels at 0°—5° C. until the pressure developed as the result of fermentation reaches 1·5 kg./cm² (21 lb./sq. in). If the fermentation gets out of control the liquid is filtered. (b) The process of bulk champagnisation : Juice is allowed to ferment (by the agency of natural yeasts) for two weeks in closed tanks under a pressure of 3 kg./cm.² (42 lb./sq. in). Before fermentation is complete it is stopped by cooling and filtration (pulp and Seitz filters).

It will be seen that a variety of treatments of the juice are used with the object of controlling fermentation : these treatments include defecation, refrigeration, centrifugation and filtration. According to Prof. Tavernier the most efficient control of fermentation is obtained by removal of " nitrogen " by defecation. This process gives a much greater removal (40-50%) of " nitrogen " than does centrifugation or filtration, such mechanical separation of yeast removing not more than 10% of " nitrogen " as a maximum. Control of fermentation by the removal of " nitrogen " (by defecation) is considered to give ciders which are less liable to subsequent disorders than ciders in which fermentation has been checked by mechanical removal of yeast. To obtain satisfactory defecation it is essential, as already stated, to use apples at the right stage of maturity, i.e. at full maturity.

*Bottling.* At one factory Cidre Doux is apparently Seitz-filtered into bottle but is not entirely free from yeasts and a maximum number of 0-5 yeasts per ml. of cider is tolerated. Other ciders are flash-pasteurised at 95° C. for 10 seconds in a layer 1·2 mm. thick, cooled with water to 25° C. and then either bottled or stored temporarily at 0° C. Flash pasteurisation is much used in this factory. Sulphur dioxide (100 p.p.m.) appeared to be used as an aid to stability in the transport of Cidre Doux to other bottling firms.

**The Ciders.**

A wide range of ciders of varying degrees of sweetness are made at the two factories visited. Some of the ciders tasted were blends of cider and unfermented apple juice; they resembled cider rather than unfermented juice. The ciders were dark in colour as compared with English or Swiss ciders and were mainly of the bittersweet type; acidity was usually rather low. They had, however, a cloying effect upon the palate, presumably due to pectin, and were in general rather too sweet for English taste. English and French ciders differ rather greatly from each other and comparison is therefore difficult.

**Cider-Brandy.**

A large volume of cider is distilled in France. At one factory the distilling plant consists of copper vessels which are heated over a naked fire. The distillate is stored in oak vats for maturing.

**General.**

As the time spent in France was short, the knowledge gained of the French methods of cider-making was less complete than that gained of the Swiss methods. A detailed comparison of the methods of cider-making employed in the two countries will not therefore be attempted. It may, however, be stated that the methods of cider-making as at present practised in these two countries differ very greatly, both in the processing of the juice prior to fermentation, and in the fermentation processes employed. These wide differences in processing are reflected in the varied nature of the ciders produced in the two countries.

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# NOTES ON APPLE JUICE PRODUCTION IN SWITZERLAND AND FRANCE.

By A. POLLARD.

The apple juice industry in some other countries was already well established in the years before the War, at a time when the industry in this country was in its early stages of development. In the intervening period the growth of our own industry has necessarily been restricted and it is only recently that expansion has again become possible. In Switzerland, where the production before the War was already very considerable, the normal developments and improvements in technique have been able to continue, and in order to obtain some insight into present day methods of production the writer was granted a Travelling Fellowship to visit juice factories and research establishments in that country.

Two weeks were spent in Switzerland in the late spring of 1948 and the first part of the time was spent with the party of British Cider-Makers who were making a tour of cider and juice factories in the Zurich area. A description of this tour and of the cider-making practices seen during the visits is given by Dr. Challinor elsewhere in this Report (p. 216). A further time was spent in visiting research laboratories including the Research Station at Wädenswil and on the return journey visits were made to cider and juice factories in France, to research laboratories in Paris and to the Cider Research Station at Rennes. At the time of the visits the pressing season was over, but it was possible to observe some of the later processing operations and to discuss the general methods of production with the Staffs of the factories.

The early history of the apple juice industries in some European countries has been described in previous Annual Reports (1937, 1938) and the present account will therefore be confined mainly to the more recent developments which were noted during the visits.

## SWITZERLAND.

### Present Position.

The apple juice industry in Switzerland now appears to be relatively stabilised, both in its extent and in the methods of production. Since the early days, following the anti-alcohol legislation in 1930-32, the industry has shown a continual expansion up to recent years and, whereas in the 1920's less than 1% of the apple and pear crop was used for unfermented juice products, in 1947 the figure approached the 10% level. According to Dr. Luethi of Wädenswil the production of juice is now equivalent to about half the cider production, the volume of unfermented juice in 1947 being about 9 million gallons against 17 million gallons of fermented cider. This expansion has been due in no small part to the work of fruit marketing organisations and to the development of the Co-operatives which have centralised much of the small-scale fruit processing. Production now appears, however, to have reached a more stabilised level and efforts are being made to adjust the supply of fruit to present requirements rather than to expand juice production further. To this end it is being recommended that the less profitable lower grade fruit be eliminated by the removal of surplus trees and by improved orchard management. The restricted export of processing fruit makes this retrenchment particularly

necessary in the supply of lower grade pears which have suffered a diminution in value due to changes in consumer taste and to the emphasis on unfermented juice rather than on distilled products. There is a less extensive use of sweet pear concentrates as sugar substitutes since the War and the limited export facilities are no longer capable of absorbing a sufficient quantity of the surplus. Some pear juice is used for blending with apple juice, but too great an admixture is apparently not favoured by the consumer.

The present production of unfermented juice in Switzerland, viz. about 9 million gallons in a country of 4½ million inhabitants, may be contrasted with the far smaller production (less than ½ million gallons) in the U.K. with a population ten times greater. The average annual production of apples in the two countries is much the same and according to the publication of the Commonwealth Economic Committee (H.M. Stationery Office, 1949) was about 470,000 tons over the years 1944-47. In addition to this tonnage of apples Switzerland produced over 300,000 tons of pears annually over the same period. With the much larger population in the U.K., and consequently a larger demand for dessert and culinary fruit, it would not be possible to expand juice production to a figure approaching that of Switzerland, but at the same time it would seem that production could be increased considerably above its present level, which now corresponds to less than one per cent of the total crop.

**Products.**

One of the main reasons for the popularity of apple juice in Switzerland is the universally high quality of the product available. In a Report on visits made in 1931 it was stated that four different methods of production were seen in operation. In the present visits the method of production appeared to be standardised and the only important variation was in the final method of stabilisation, the juices being either filtered sterile into bottle or pasteurized in bottle. The final products were all very similar in general character although they varied somewhat in composition due to the addition of variable amounts of pear juice or of juice concentrate. The methods of juice storage and of stabilisation were frequently indicated on the labels, as was also the type of fruit used in manufacture. The term "apple juice" was required to be used only for the pure apple products, the mixed apple and pear blends being described as "fruit juice."

Many of the products were sampled at the factories and some were bought elsewhere. They differed in composition (apple juice as against apple and pear blends), in degree of carbonation and according to the method of stabilisation used. They were, however, all very similar in their general character. They were invariably brilliant and showed no deposit ; carbonation was usually slight. A few samples had a definite pasteurized taste, but as a rule this was slight and it was sometimes difficult to detect whether the juice had been sterile filtered or pasteurized. The products were sweet and fairly low in tannin, but were sufficiently high in acidity to give a balanced flavour. According to Dr. Luethi, most manufacturers aimed at producing a standard product and the fruit or juice is blended to give a final acidity of 0·5% malic acid. Excess acidity may be corrected by the addition of pear juice or of suitable juice concentrate.

In all the factories visited apple juice was by no means the only product made : the manufacture of juice and cider went on side by side and production could no doubt be directed to one or other product as the occasion made

necessary. The production of concentrates enabled any fruit surplus to be stored with economy of space until required. It was evident that a full utilisation of pomace was being made, the factories being provided with pomace driers capable of dealing with their own pomace and with pomace brought into the factory from outside. The production of high quality apple pomace has encouraged the development of the pectin industry and of a variety of pectin products. Pectin was being extracted in one of the factories visited and concentrated before drying to a stable powdered product. Among other products made in the factories were unfermented grape juices, distilled liquors and cider vinegar. In some factories, and particularly in the Co-operative concerns, the storage and distribution of fruit and vegetables and the preparation of dehydrated products provided an extension of the working season and enabled a large nucleus of workers to be retained throughout the year.

**Methods of Production.**

Pressing operations were over at the time of the visit, but it was possible to obtain a general view of the factories and of the lay-out of the plant; some of the later juice processing was still being carried out. It was emphasised that only good quality fruit is used for unfermented juice, but even so, washing operations are thorough. In one factory the fruit is passed by conveyor belt under a series of high-pressure water jets, on its way to the mill. The mills seen were all of the Bucher-Guyer type in which the fruit is thrown by a rotor against a series of fixed serrated knives and this type of mill would appear to have replaced the older forms described in earlier Reports. In some factories the milled pulp is transported to the press by pump, whereas in others the mill is situated directly over the press. The method used seemed to depend largely on the general lay-out of the factory. There would appear to be little change in the method of pressing, the pulp being usually subjected to two pressures in a swivel press without any intermediate pomace breaking. The first pressing is at about 100 lb./sq. in. and the second at about 350 lb./sq. in.

**Juice Treatment.**

Some unclarified juice is sold direct from the press for immediate consumption but only clarified juices were seen in bottle. After coarse filtration the juice is clarified by enzyme treatment and fining with gelatine. It is then filtered to storage tanks using the usual type of plate filter or asbestos pulp filter. The earlier method of storage under carbon dioxide at 8 atmospheres pressure and at normal cellar temperature was seen in several factories, but a trend towards lower temperature storage was generally apparent. In the newer storage cellars juice was being kept either under 3 atmospheres pressure at 0—1° C. or at this temperature without carbon dioxide pressure but under sterile conditions. It was stated that the lower storage temperature gave a superior product. If stored under pressure the enzymed juice is given a kieselguhr or pulp filtration, whereas if stored under sterile conditions it receives a further sterilizing filtration before filling into the storage tanks. If the juice is to be filtered sterile, the tanks themselves are first given a thorough cleaning and are then sterilized by treatments with formaldehyde and with sulphur dioxide before use. Some of the storage cellars were very extensive and of impressive appearance, the largest being capable of holding 250,000 gallons of juice at a temperature of 1°C. The tanks were of mild steel or aluminium alloy lined with Ebon or Gashell.

**Bottling.**

From the storage tanks the juice is given a further filtration, the type of filter being dependent on the method of stabilisation to be adopted. Juice for pasteurization is filtered to brilliance, while juice sent out without pasteurization is given a further sterile filtration before filling into sterile containers. Cold filtration to bottle was only seen in one of the factories visited, but it was understood that several other factories are using this process. In the other factories the bottled product is sent out after pasteurization, although some juice is sent out in bulk after sterile filtration into glass demijohns (6 gallons) or into small casks of wood or aluminium. The juice sent out in this latter manner is intended for quick consumption and the method is apparently not considered entirely satisfactory.

(a) *Hot process.* The juice for pasteurization in bottle is freed from the greater part of the carbon-dioxide, filtered brilliant and bottled cold. No flash pasteurization was seen in any of the factories visited. Some juice is put up in bottles with crown cork closure, but more usually in the spring cap type of bottle in which the seal is effected by a porcelain stopper carrying a rubber washer. Two layers of bottles are loaded into a trolley which is then wheeled into the sterilizing compartment where hot water in constant circulation is sprayed over the bottles. The temperature is raised to 68° C. in twenty minutes and after a holding period of twenty minutes, the bottles are cooled during a similar period.

In one factory juice was being filled cold into large glass demi-johns and was pasteurized by inserting electrodes through the neck of the container, the temperature being eventually raised to 76° C. After removal of the electrodes the vessel was sealed by a cap fitted with a sterilized filter pad. This method of storage was stated to be rather unsatisfactory and it is not known how far the flavour of the juice was affected by the treatment.

(b) *Cold process.* In one factory sterile bottling was in process at the time of the visit. The bottles, after rigorous cleaning by soaking and brushing, were sterilized by passage through a steam chest where they were heated to 90°—95° C. for an hour. They then passed into the bottling chamber where an excess pressure of one tenth atmosphere of filtered air was maintained. One operator was in charge of the filler and each bottle when filled was closed manually and passed out of the chamber. The plant had a capacity of 10,000 bottles per 10 hour day. A careful check on the sterility of the product was maintained by incubating a sample of each run for 2 to 3 weeks at 35° C. and furthermore, all bottles were kept at room temperature for observation over 3 to 4 weeks. It was stated that only one or two bottles in each day's run were found faulty if the plant was efficiently sterilized before bottling was begun.

**Concentrated Juice.**

A very considerable volume of juice is converted into concentrate, and apple and pear juices were being concentrated in two of the factories visited. One factory was using a multi-stage evaporator utilising water cooling of the vapour, while the other was using the heat-turbine system in which the temperature of the vapour is raised by a compressor and the heat developed is further utilised for juice evaporation. In both types of plant the evaporation was carried out at low temperature (35° C.) and six to seven-fold concentration. No collection of volatile ester fraction was apparently made and the products seen were devoid of any characteristic apple or pear aroma. Both apple and

pear concentrates were golden brown in colour, sweet and attractive in flavour and were very similar in character. It was understood that some sweet concentrate is made by de-acidification, the malic acid being separated as the calcium salt by methods similar to those worked out at Long Ashton during the War years.

## FRANCE.

### Present Position.

The production of apple juice in France has shown an increase in recent years, but the expansion has not been comparable with that found in Switzerland. According to the Report by Charley in 1937, unfermented apple juice was, in that year, not yet on the market, although some installation of storage plant had begun and juice was already being made and stored for blending with cider.

In France apple juice has to meet competition with wine, beer and cider, with other fruit juices such as grape juice, and more particularly with citrus products imported from the colonial possessions. Furthermore the tendency to produce naturally sweet ciders in place of the rougher type of cider provides a beverage which may attract some sections of the public who would otherwise be consumers of the unfermented product. In spite of this, and although production is still limited, apple juice appears more generally available and well-known than in this country : the increasing interest which is being shown in research on fruit juice problems suggests that a further expansion in production is probable. This would provide a further outlet for the very large tonnage of fruit which is produced, France being second only to the United States in apple production and about 90 per cent of the fruit being of cider type.

### Products.

It was not possible in the short time available to obtain a complete picture of the methods used in apple juice processing, but it was evident that many types of product were being made and that production has not yet become standardised as in Switzerland. The products sampled in the factories were attractive in appearance and flavour and were of high quality, but juices bought in the open market were very variable in type and quality. Some of the latter were clear and of attractive appearance and flavour, while others were cloudy, some bottles carrying heavy and unsightly deposits. The emphasis which has always been laid in France on the importance of " body " or fulness in drinks has discouraged the use of the methods for juice clarification and filtration which have been followed in other countries. The slightly cloudy and pectinous type of juice has therefore been more popular than the brilliant but thinner product favoured in Switzerland or in this country.

The difference between these two types of juice is further brought out by differences in the fruit used, a greater proportion of true cider types being used in France. Some culinary apples are imported for blending purposes, but the bulk of the fruit used appears to be cider fruit of relatively low acidity. A considerable volume of apple juice is made for blending with fermented cider to give a range of sweet ciders (Cidre doux) and this method can be used as an alternative to the process whereby naturally sweet cider is made by arresting fermentation at an early stage. The methods by which this is effected are discussed in detail in the accompanying Report on cider-making processes

and they will only be mentioned here in so far as they affect apple juice processing.

**Methods of Processing.**

In France, as in Switzerland, the manufacture of cider and of unfermented juice proceed side by side far more than they do in England and this gives a greater flexibility, in that production can be directed to one or other product. In Switzerland the methods used for apple juice production have, however, departed from those used in cider-making ; in France the two procedures often follow each other more closely.

For example, the process of maceration is still carried out in some factories, the milled fruit being allowed to stand for 5-6 hours before pressing at a comparatively low pressure (170-250 lb./sq. in), the once-pressed pomace being then exhausted by serial diffusion in tanks. The practice of maceration appears to be essential if the juice is to be clarified by keeving (defecation) : if other means of clearing are to be used then the pulp may be pressed directly. The clarification by keeving is described in some detail in the accompanying Report on Cider-Making and elsewhere in this Report, p. 216 where the underlying principles and methods are given more fully. In cider-making the chief function of the process is the reduction of the nitrogen content of the juices, and the segregation of the micro-biological flora, but from the point of view of juice clarification the process fulfils the function of both precipitating pectin and at the same time removing much of the other material in colloidal suspension. This natural pectase action, however, proceeds more slowly than the pectin breakdown brought about by the addition of commercial enzyme preparations and several days are involved in the process. The use of these commercial enzymes does not appear to be popular and as an alternative to keeving, clarification is brought about by heat ; the juice colloids and fine suspended matter are coagulated by flash pasteurization and this is followed by centrifugation. This procedure leaves pectin in solution but removes much of the material liable to cause deposits on storage.

Refrigerated storage was in general use, the juice being maintained without gas pressure at 0° C. in large tanks lined with glass or a wax bitumen composition. The clarified and filtered or centrifuged juice is passed directly through a cooling plant, or after a preliminary flash pasteurization, and filled into the storage tanks at a temperature of 0° C. The tanks were fitted with internal cooling coils and this method of refrigeration was more common than the type of space-cooling seen in the Swiss cellars. The methods of storage used in France appear less rigorous than those found necessary in the Swiss factories and it is probable that the type of fruit and the methods of juice clarification furnish a juice of lower nitrogen content and one which is thus less liable to undergo fermentation.

Much of this juice is subsequently used for blending with fermented ciders or for later fermentation, but some is bottled as unfermented juice and stabilised by pasteurization. No cold-processed juice was seen during the visits. It would seem that, although much of the unfermented juice made is blended to give sweet ciders, the output of the unfermented product could be readily increased without any great modification of plant or processes.

# THE CLARIFICATION OF APPLE JUICE.

## THE ACTIVITY OF PECTASE IN SOME APPLE VARIETIES.

By MARGARET E. KIESER, A. POLLARD and AUDREY M. STONE.

The preparation of a clear apple juice is usually brought about by the removal or partial removal of the pectin present, both to facilitate filtration and to remove at the same time other material in colloidal suspension which may give rise to deposits on subsequent storage. In this country, in Switzerland and in the United States this is commonly carried out by the addition of pectin degrading enzymes which usually contain both pectase (pectin demethoxylase) and pectinase (polygalacturonase). The action is essentially the cleavage of the pectin molecule by the pectinase present in the enzyme preparation. As the colloidal system in the juice is stabilised by the pectin, the breakdown need only proceed to an extent sufficient to upset the colloidal equilibrium : the pectin left in solution and estimated as calcium pectate may still be very considerable.

A method of juice clarification which is independent of added enzyme is involved in the process of " defecation " or " keeving." This cider-making procedure, which was more extensively practised in this country in the past, is still used in France for cider and juice manufacture. As described in the accompanying Reports (1, 2), its purpose is not merely to remove pectin, but to precipitate nitrogenous compounds and micro-organisms at the same time. The application of the process in cider-making has been described in an earlier Report by Charley (3) who saw the process in operation in France on one of the earlier Cider-Makers Tours and was stimulated to carry out some practical tests on his return. The process is based on the precipitation of the juice pectin in the form of pectic acid or calcium pectate following the action of natural pectase (pectin demethoxylase) present in the juice itself : the conditions under which the process takes place have been described by Warcollier and in the later publications of Tavernier (e.g. 4).

The process is commonly carried out with cider apple juices and it is generally assumed that such natural pectase action plays little part in the usual clarification methods used for unfermented apple juice (c.f. Weber and Deuel (5)). The optimum pH for pectase action lies near neutrality and the pH values of most apple juices, particularly those of the culinary and dessert varieties used for apple juice production, lie well below this. In cider varieties, however, the mechanism is sufficiently active to bring about a flocculation and clarification in the course of a few days under favourable conditions and this may occur at a pH below 4 as shown by Le Corvaisier (6).

In the tests made by Charley (3) the keeved juices showed a marked fall in soluble pectin, the extent of the change varying according to the type of juice and the temperature. Later tests (7) showed that this type of pectin removal normally occurs in English ciders, for after fermentation the pectin which can be precipitated as calcium pectate is practically negligible, the removal of pectin during fermentation being associated with the presence of pectase in the fruit rather than with yeast action. The efficiency of the pectin precipitation and juice clarification during the keeving process has been shown by Bejambes and Tavernier (8) to be dependent on an adequate concentration of calcium in the juice, and, in practice, a mixture of calcium

carbonate and sodium chloride is frequently added. The calcium carbonate facilitates the precipitation of calcium pectate while the sodium chloride enhances the activity of the pectase present.

The role played by apple pectase is therefore of importance in normal cider-making, but it may also play some part in the clarification of unfermented juices. Furthermore it may also affect the quality of pressed pomace which is intended for subsequent pectin extraction, for, as described in the Report by Hinton (9), pectase action occurring spontaneously in fruit tissue may not only reduce the amount of soluble pectin which can be extracted, but may also considerably modify the jellying properties of the pectin, and to an unpredictable degree. Some exploratory tests have therefore been made on a number of apple varieties to obtain further information on the occurrence and activity of pectase in some common apple varieties and its effect on juice clarification and pectin quality.

**Methods.**

Measurement of pectase activity in many plant tissues, such as tomato, is simplified by the high level of activity shown and by the ease with which the enzyme can be concentrated : in apple tissue the activity is much lower and methods for purification of the enzyme require further investigation. The methods for testing pectase activity include the following :—

(1) The precipitation of pectin from solution as pectic acid or as calcium pectate,

(2) the formation of a pectate gel at a suitable pH,

(3) the estimation of liberated carboxyl or methyl groups from the pectin,

(4) the changes in pectin quality following the action of the enzyme. In the present tests some use has been made of all these methods.

**Source of Material.**

The pectase in apple tissue is assumed to be associated mainly with the insoluble pomace fraction, as evidenced by the use of maceration in the preparation of juice intended for keeving and, as will be shown later, this has been confirmed in the tests to be described here. Minced apple tissue or pressed pomace was extracted with water or with phosphate buffer at various pH values and the extracts were tested for pectase activity on pectin solutions at pH7, the hydrolysis being measured by electrometric titration of the liberated carboxyl groups.

Extracts of whole fruit of immature Bulmer's Norman, Dabinett and Monarch made at pH 7 showed negligible activity over a period of 24 hours. A preparation of tomato pectase when tested in this manner gave a 50% hydrolysis of methyl ester groups in 5 minutes. It appeared probable that the inactivity of the apple preparations was associated with the immaturity of the fruit and a mature sample of Morgan Sweet was therefore tested. Pomace extracts made at the pH of the fruit (4·2) or after the addition of sodium hydroxide (to pH 5·6) showed considerable activity, the extract made at the higher pH giving practically complete hydrolysis in 24 hours. A sample of macerated juice of this variety after the addition of defecating salts (0·03% $CaCO_3$, 0·04% NaCl) showed an almost complete separation of the juice pectin from solution on standing overnight, the pectin being thrown down as a copious white gelatinous precipitate (original calcium pectate 0·09%, final 0·02%).

The effect of fruit maturity was shown by further tests on Dabinett

at different dates. When the fruit was practically mature pomace extracts were made at pH values of 4, 6 and 8 in phosphate buffer and these were added to pure pectin solutions (powdered apple pectin, grade 300°). The extracts made at the two higher pH values showed activity, and when added to a pectin solution at pH 8 the solutions set to a gel overnight. The control solutions containing pectin or enzyme alone did not gel. Estimation by the method of Holden (10) of the methyl alcohol liberated indicated a hydrolysis equivalent to about 35% of the maximum possible in the solution containing pectase prepared at pH 6 : the extract prepared at pH 8 showed a lower figure at 13%.

Some other cider apple varieties were tested in this manner viz., Woodbine, Yarlington Mill, Slack-ma-Girdle, Frederick, Kingston Black and Medaille d'Or. When tested against pectin at pH 8 the mixtures gelled overnight, the pectin being de-esterified to the extent of 20-30%. At pH 6 only viscous solutions were produced.

Although apple tissue thus shows pectase activity over the pH range 6-8, this is well outside that normally found in the tissue itself. Further tests at pH values comparable with those of the juices themselves were therefore carried out and these tests were combined with others being made at the same time by the Cider Section on the effect of keeving on fermentation. These latter experiments will be described at a later date.

**Pectin changes in keeved juices.**

In the above experiments (Cider Section) Dabinett juices, both freshly pressed and after maceration with pomace, had been put up with and without the addition of defecating salts (D.S.). Samples of these juices were taken for pectin estimation and in addition a further sample of the macerated juice with salts (B/D) was preserved with sodium benzoate (0·125%) and a portion of this was filtered clear through pulp at the start of the experiment (Juices B/D/Bz and B/D/Bz/F respectively). The figures for initial pectin in these juices are shown in Table I, together with those obtained after standing at cider-house temperature for 11 days, the values being expressed as calcium pectate (the pectin being first precipitated from the filtered solutions with acetone). At the end of this time fermentation had begun in all but the benzoated samples and heavy pectin clots had formed in the juices to which defecating salts had been added. It may be seen from the Table that this addition when made to the unmacerated juice (A/D) or to the macerated juice (B/D) was followed by a fall in soluble pectin, the juices A/C and B/C without the addition showing little change.

TABLE I.

Pectin Changes in Apple Juice During Keeving.

| Juice treatment | pH | Calcium pectate % Original | Final |
|---|---|---|---|
| A/C Fresh pressed .. | 4·42 | 0·081 | 0·068 |
| A/D „ „ + D.S. .. | 4·66 | „ | 0·021 |
| B/C Macerated .. .. | 4·46 | 0·099 | 0·100 |
| B/D (a) „ + D.S. .. | 4·70 | „ | 0·015 |
| B/D (b) „ „ .. | 4·70 | „ | 0·018 |
| B/D/Bz „ „ + sodium benzoate .. | 4·70 | „ | 0·014 |

That this precipitation of pectin was not directly the result of fermentation is shown by the similar change in the benzoated juice B/D/Bz. In Table II are given further pectin analyses of this juice made after filtration at intermediate dates.

TABLE II.

PECTIN CHANGES IN JUICE PRESERVED WITH SODIUM BENZOATE.

| Juice | Day | Calcium pectate % |
|---|---|---|
| B/D/Bz .. .. | 0 | 0·099 |
| „ .. .. | 1 | 0·107 |
| „ .. .. | 2 | 0·107 |
| „ .. .. | 5 | 0·099 |
| „ .. .. | 7 | 0·075 |
| „ .. .. | 11 | 0·014 |

There was no fall in soluble pectin until after the 5th day, after which it was gradually thrown out of solution, the amount finally remaining in solution being about 14% of that present originally.

Although the pectin remained in solution until about the 5th day, the analysis of samples of pectin precipitated before that date showed that some degradation had already taken place. Aliquots of the benzoated solution B/D/Bz and of the filtered solution B/D/Bz/F were taken at intervals and the pectin precipitated by the addition of acetone to 60%. Saponification of these preparations showed that there was a gradual fall in methoxyl value from the first day until the pectin was thrown out of solution, as shown in Table III.

TABLE III.

| Day | $OCH_3$ as % of calcium pectate value. |
|---|---|
| 0 | 10·8 |
| 1 | 8·5 |
| 2 | 8·1 |
| 7 | 6·4 |
| 11 | 5·0 |

These changes were accompanied by changes in the physical appearance of the pectin, which became less " stringy " and increasingly granular in appearance. It is thus apparent that considerable degradation of pectin can occur as a result of pectase action over the pH range 4-5 and that the effect may be considerable even during the course of the first day.

**The effect of pH and variety.**

To obtain further information on the effects of juice pH and fruit variety, a series of small scale tests was made on macerated juices of the varieties listed in Table IV. The two dessert varieties were over-mature at the time of the experiment (January) but all fruit had been kept in cool store and was in good condition and free from any visible rot or damage. For the purpose of comparison, a mixed sample of badly rotted fruit was selected and included in the test. The fruit was washed and milled and the pulp allowed to stand

## TABLE IV.

### Pectase Activity in Apple Varieties.

| Variety | Juice pH | Testing pH | Viscosity time in seconds | | Calcium pectate % | | Observations after | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Original | Final | Original | Final | 1 day | 2-3 days | 5-7 days |
| Cox .. .. .. .. | 4·12 | 4·1<br>4·62 | 498 | 73<br>52 | 0·218 | —<br>0·045 | n<br>G | g<br>GS | G<br>GS |
| Rival .. .. .. .. | 4·24 | 4·0<br>4·60 | 516 | 62<br>51 | 0·274 | —<br>0·048 | n<br>G | g<br>G | G<br>GS |
| Chisel Jersey .. .. .. | 4·36 | 4·0<br>4·67 | 376 | 104<br>70 | 0·268 | 0·216<br>0·085 | n<br>g | v<br>G | v<br>G |
| Bedan .. .. .. .. | 4·62 | 4·0<br>4·5<br>5·2 | 152 | 97<br>63<br>63 | 0·176 | 0·090<br>0·044<br>0·037 | n<br>v<br>v | v<br>g<br>g | v<br>g<br>GS |
| Reine des Pommes .. .. | 4·11 | 4·0<br>4·45 | 205 | 74<br>73 | 0·187 | 0·153<br>0·157 | n<br>n | n<br>n | n<br>n |
| Slack-ma-Girdle.. .. .. | 4·22 | 4·0<br>4·64 | 316 | 123<br>84 | 0·209 | 0·154<br>0·103 | n<br>v | v<br>v | v<br>v |
| Bramley .. .. .. .. | 3·30 | 3·38<br>3·96<br>4·52 | 352 | 238<br>92<br>74 | 0·206 | 0·211<br>0·200<br>0·174 | n<br>n<br>n | n<br>n<br>n | v<br>v<br>v |
| Michelin .. .. .. .. | 4·40 | 4·02<br>4·48 | 349 | 104<br>64 | 0·199 | —<br>0·088 | n<br>v | n<br>v | v<br>v |
| Woodbine .. .. .. | 4·32 | 4·01<br>4·51 | 467 | 125<br>67 | 0·231 | —<br>0·209 | n<br>v | n<br>g | v<br>G |
| Tardive Forestier .. .. | 4·44 | 4·01<br>4·48 | 680 | 355<br>216 | 0·217 | —<br>0·240 | n<br>v | n<br>v | v<br>v |
| Upright Medaille d'Or .. .. | 4·72 | 3·96<br>5·18 | 330 | 111<br>67 | 0·184 | —<br>0·033 | n<br>G | v<br>G | v<br>GS |
| Yarlington Mill .. .. .. | 4·52 | 4·05<br>4·43 | 1078 | 68<br>58 | 0·287 | —<br>0·035 | v<br>G | G<br>GS | G<br>GS |
| Brown rots .. .. .. | | 4·51<br>4·62 | 146 | 59<br>59 | 0·237 | —<br>0·046 | n<br>n | v<br>G | v<br>G |

n = no visible change ; v = viscous ; g = slight gel ; G = strong gel ; S = separation of clear liquid.

overnight before pressing. After pressing the juices were treated with defecating salts to ensure the presence of sufficient calcium for calcium pectate gel formation and they were then allowed to stand in bottles at room temperature.

The original juice pH values lay mainly between 4·0 and 4·5 and the addition of the calcium carbonate raised the pH by about 0·3 -0·4 unit. The juices were therefore adjusted arbitrarily to pH 4·0 and 4·5 at the beginning of the test, the precise values being given in the table. Samples of each juice were filtered at the beginning and end of the test and determinations made of relative viscosity and of calcium pectate after acetone precipitation.

As indicated in the table, the juices showed considerable differences in behaviour, some varieties such as Cox, Rival, Yarlington Mill and Upright Medaille d'Or set to firm gels overnight and eventually gave a clear separation of solid and liquid phases. Others showed a slow gelation (Bedan and Woodbine) while others became viscous or showed no obvious change. These effects are reflected in the figures for calcium pectate, the soluble pectin in the gelled juices being reduced to very low values. The fall in viscosity of the filtered solutions similarly followed the fall in soluble pectin, the most striking change being shown by Yarlington Mill which initially had a very high viscosity. Even where the soluble pectin as estimated by calcium pectate showed little change (Reine des Pommes, Bramley and Tardive Forestier) the fall in viscosity indicated a lowering of pectin quality.

The effect of pH on the rate of pectase action is brought out by the viscosity changes in all varieties at the two pH values and by the calcium pectate figures for Chisel Jersey, Bedan and Slack-ma-Girdle. At the lower pH the drop in viscosity, although considerable, is less than at the higher value. In the Bramley juice there is no appreciable fall in soluble pectin (calcium pectate) even at the higher pH although the viscosity shows a fall at both pH 3·96 and pH 4·52. At the normal juice pH (3·38) the fall is much less.

These pectin changes show no apparent relation to the onset of fermentation which became noticeable on the third day, for fermentation occurred in all samples irrespective of the changes in viscosity or soluble pectin. Furthermore, in the previous experiment the benzoated juice which remained free from fermentation showed changes similar to those which fermented. Nor can the changes be readily ascribed to the action of mould enzymes, as the fruit was free from any visible damage. In the juice from the rotted fruit the pectase action was less strongly marked than in the other juices, although it should be remarked that the pectin in this former juice had already suffered some change as shown by the low initial viscosity : the calcium pectate figure was, however, fairly high.

While these tests show that pectase action is associated with many apple varieties, it is evident that the clarification which can be brought about is usually slower than that which can be produced by the addition of commercial enzymes and requires a pH not lower than 4 to 4·5. Furthermore, the precipitate thrown down by pectase action is more bulky and difficult to separate than that which follows the polygalacturonase action of the added enzyme preparations. The natural pectase action may, however, influence the rate of clarification produced by added enzyme and it may materially affect the quality of pomace which is subsequently to be used for pectin extraction. It is intended to give further attention to these points in later work.

## SUMMARY.

A preliminary test has been made of the pectase activity of some apple varieties by following pectin changes taking place in tissue extracts and in juices.

In juices undergoing the process of defecation or keeving, the precipitation of pectin from solution is preceded by demethoxylation. Cider apple juices exhibit this type of clarification to a variable degree and the effect is also shown by some other varieties.



## ACKNOWLEDGMENTS.

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# THE ENZYME SYSTEM AND BROWNING OF PROCESSED PEARS


By DILYS JAMES and ALICE CRANG.


A number of complaints have been received in the last few years that bottled or canned pears have turned brown rapidly on opening. Examination of one of the samples concerned showed an abnormally high count of viable bacterial spores, but no yeasts or moulds and no sign of fermentation. This sample had been processed in a domestic pressure cooker. Other reports of browning were concerned with bottled pears processed in water by the method which has been recommended for some years (Method I). The fruit otherwise appeared to be quite normal. This led to an investigation of the enzyme system concerned in browning in relation to the maximum temperature reached by the pear tissue during different methods of processing.

## EXPERIMENTAL.

Most of the work was carried out on William's Bon Chretien pears, but Comice, Conference and Bristol Cross were also tested and found to brown readily when cut surfaces were exposed to the air. Other varieties which showed less tendency to brown, and were also of poorer canning quality, were not used.

The pears were tested at four stages of ripeness :—

A. Under-ripe, skin green, flesh hard and no juice on cutting.
B. Slightly under-ripe, green of skin just turned to yellow but fruit still hard and little juice on cutting.

C. "Canning ripe"—Skin yellow, flesh just firm, but juicy.
D. Slightly over-ripe. Skin deep yellow, flesh soft and very juicy.

**Chemical Methods.**

(a) The total tannin was determined in the pear tissue by the potassium permanganate reduction method. One series was also judged by the chromatograms.

(b) The "catechol tannin" was tested by the green colouration developing with ferric chloride (1) changing to purple on the addition of sodium carbonate (2).

(c) The presence of oxidase was shown by a blue-purple colour with a 1% alcoholic solution of benzidene or a deep red colour with 1% alcoholic solution of guaiacol. The latter reagent was preferred and used in later tests.

(d) Peroxidase was demonstrated by tests (c) above on the addition of hydrogen peroxide.

(e) The tests for peroxide were :—

(i) Blue colouration with potassium iodide and starch, for hydrogen peroxide.
(ii) Blue colouration as (i) on the addition of acid, for organic peroxide.
(iii) Blue colour in the ether layer with potassium dichromate, sulphuric acid and ether.

(f) The rate of browning in the pear tissue was timed with a stop watch from the moment of cutting the fresh pear or of opening the tube or container of processed pear. The degree of browning was judged by eye.

(g) For testing the temperature of enzyme inactivation, the pears were first cooled in a refrigerator and macerated on ice. The pulp was quickly transferred with a pipette into test tubes ($2'' \times \frac{3}{8}''$) to a depth of half an inch. Corks were loosely inserted and the tubes heated in a water bath with a thermometer inserted in the pulp in one of the tubes. The temperature was raised from 100° to 210° F. in thirty minutes, the sample tubes taken out at intervals and cooled in iced water. The enzyme activity in the processed fruit was compared with that in the fresh pear tissue by means of tests c, d, e and f, having shaken the pulp on to a white tile for the last test.

(h) The temperatures during processing in cans and preserving jars were tested by means of a thermo-couple, having the probe inserted in the pear tissue in the centre of the container.

**Processing Methods.**

The pears at the selected stage of ripeness were peeled and halved, using a stainless steel knife, and cores removed with a silver spoon. They were at once immersed in salt water (2 oz./gal.), while sufficient fruit was prepared for two or three containers. The pears were then rinsed quickly in cold water, packed at once into the container (22 oz. per jar and $18\frac{1}{2}$ oz. per can) which was filled immediately with 30° Brix syrup. After processing jars were cooled in the air, and cans in cold water.

I. 2 lb. glass jars were used, filled as above with fruit and cold syrup. They were immersed in a water bath which was raised from cold to 190° F. in $1\frac{1}{2}$ hours and maintained for 20 minutes.

II. Similar warmed jars were filled with fruit and boiling syrup. The jars were processed in a domestic pressure cooker containing one inch depth

of water heated to boiling point before adding the jars which were raised on a rack just clear of the water. The cooker was closed without exhausting the air, and the jars processed for 12 minutes at 5 lb. per sq. inch pressure registered. This was the processing time given to one of the original cans received. Variations in the times and pressures used for processing were also made.

III. "A2½" size fruit lacquered cans were filled with the pears and boiling syrup, sealed at once, and processed in boiling water for 21 to 26 minutes from the start, depending on the time taken for the water to reboil.

IV. The fruit was placed in jars as in I, and the fruit heated for 1½ hours in an oven at 240° F. The jars were then quickly removed and filled with boiling sugar syrup.

## RESULTS.

### Tannin Content and Browning of Raw Pears.

The pears tested varied considerably in the tendency to turn brown on slicing. These variations were not only in different varieties, but also with their origin and degree of ripeness. For instance, some William's Bon Chretien pears imported from S. Africa gave very little catechol tannin reaction, whereas those grown in this country contained some of the highest amounts of this constituent.

The tannin content, as determined with potassium permanganate, increased with ripening up to stage B, then decreased as the fruit became fully ripe and over-ripe. The catechol tannin, as tested with ferric chloride and sodium carbonate, invariably showed increase up to stages B and C, then lessening as the fruit became fully ripe. This was confirmed by a study of the chromatograms of one series, in which the tannins were in descending order, C, B, D, A. In this examination fructose, glucose and sucrose were also identified, the sugars being greatest at stage C and least at A, but no other phenolic or reducing substances were found.

### Enzyme Activity and Degree of Browning.

Tests were made on the time taken for the fruit to brown on exposure to the air when sliced and pulped. The results on the same pear were :—

Slices—light brown in 30 minutes, benzidene test purple in 5 minutes.

Pulp—dark brown in 5 minutes, benzidene test purple in 1 minute.

Juice—dark brown in 10 minutes, benzidene test no change until 30 minutes.

The fresh pear pulp tested for peroxide gave positive results in acidified pear-pulp only, showing peroxide to be present in an organic form.

Tests with benzidene were carried out as follows :—

(i) Benzidene alone : A gradual purple colouration, the stone cells turning orange.

(ii) Benzidene + $H_2O_2$ : Bright blue colouration at once, the stone cells developing this colour first.

(iii) Benzidene + $KMnO_4$ : Result as (i) but immediate colour.

(iv) Benzidene + $K_2Cr_2O_7$ : Result as (i) but stone cells blue.

Therefore the oxidation of benzidene in the presence of pear pulp is catalysed by potassium permanganate and hydrogen peroxide, and less effectively by potassium dichromate.

The colour given with potassium permanganate was identical with that occurring in oxidation by pear tissue only. A different colour was obtained

with hydrogen peroxide, giving evidence that the peroxide in the enzyme system is not hydrogen peroxide.

**Effect of Heat on the Enzyme System.**

Pear pulp was heated in a water bath (method (g)) and tests for oxidase and for peroxidase were made on replicate samples.

*Oxidase.* Browning was observed to occur at an increased rate in pear-pulp that had been heated to 120° F., and samples removed at higher temperatures browned even more rapidly, until at 160° F. the change was instantaneous. Above this temperature the rate declined, and after heating to 180° F. no browning took place.

Reaction to benzidene was positive up to 160° F., weakly positive up to 180° F. and negative in samples heated to higher temperatures.

*Peroxidase.* Reaction to benzidene and hydrogen peroxide was increasingly positive up to 160° F. (blue-purple in 15 seconds), the colour developing more slowly with rise of temperature (5-8 minutes at 180°; 1½ hours at 190° and 200° F.).

Further tests confirmed that oxidase activity remained up to 180° F., and peroxidase up to 200—210° F., though only weakly above 160° F.

Pear pulp heated to 190° F. and then left at room temperature for four days before opening showed no browning nor any colouration on addition of benzidene. Oxidising agents were then added :—

(i) Benzidene + 1 drop 0·1% $KMnO_4$ : Stone cells orange, remainder blue at once.

(ii) Benzidene + 1 drop $K_2Cr_2O_7 + H_2SO_4$ : Blue developed slowly.

(iii) Benzidene + 1 drop $H_2O_2$ : Stone cells blue in 1 minute, remainder in 5 minutes.

In the absence of the pear tissue (ii) turned blue slowly, but no blue developed in (i) or (iii).

Therefore atmospheric oxygen alone cannot complete the oxidation of tannin, as observed by browning or the blue colour with benzidene, but this can occur if oxygen is present in an active form.

**Inactivation of Enzyme System at Different Stages of Ripening.**

Further tests were made the following year on William's Bon Chretien pears, locally grown, at the four selected stages of ripeness.

The temperature of inactivation of the enzyme system, as tested with benzidene or guaiacol, and the degrees of browning after processing are given in Table I. The fruit was cooled in the containers before opening and testing.

It is interesting to note that enzyme inactivation occurred at a slightly higher temperature in the under-ripe fruit (stage B). No browning or enzyme activity was found in the pears which on processing attained the temperature required to inactivate the enzyme in small test tube samples.

## DISCUSSION.

**Comparison of the Processing Methods.**

I. The method of bottling pears by processing in a water bath as in Method I may not be sufficient to inactivate the enzyme system, consequently slight discolouration may occur after the bottles have been opened. It is

TABLE I.

Enzyme Reaction at Various Temperatures.

| Grade of Ripeness | pH | Temperature of inactivation of the enzyme system, °F. | Method of processing | Max. internal temp. °F. | Time to reach Max. (mins.) | Oxidase (guaiacol) test | Peroxidase (guaiacol + $H_2O_2$) test | Degree of browning after opening |
|---|---|---|---|---|---|---|---|---|
| A | 4·12 | 184–187 | I | 181·5 | 110 | + | + | + |
| | | | II | 167·5 | 37 | ++ | ++ | ++ |
| | | | III | 204 | 26 | — | — | — |
| | | | IV | 183·5 | 108 | ++ | ++ | ++ |
| B | 4·32 | 187-188 | I | 181·5 | 112 | + | + | + |
| | | | II | 184 | 23 | + | + | + |
| | | | III | 205·5 | 23 | — | — | — |
| C | 4·25 | 183 | I | 179·5 | 112 | + | + | + |
| | | | II | 151·5 | 39 | +++ | +++ | ++++ |
| | | | III | 204 | 24 | — | — | — |
| | | | IV | 169 | 108 | ++ | ++ | ++ |
| D | 4·18 | 181 | I | 180 | 110 | — | + | ++ |
| | | | II | 174 | 21 | ++ | ++ | +++ |
| | | | III | 201·5 | 24 | — | — | — |
| | | | IV | 185 | 91 | — | — | ++* |

* The browning probably occurred before the maximum temperature had been reached.

intended to recommend increasing the time of holding at 190° from 20 to 30 minutes to overcome this. In a test for this time a temperature of 186° F. was reached in the centre fruit.

II. When processing in a pressure cooker without exhausting the air, the pressure indicated will be a mixture of steam and air pressure, and the temperature may be considerably lower than that corresponding to steam pressure alone. The maximum internal temperature of the jars in the cooker under these conditions also varies as to whether the base of the container is in contact with the water in the cooker.

It was found that processing at 5 lb. pressure for 12 minutes, without exhausting the air, was quite insufficient either to inactivate the enzyme system or to ensure good keeping quality, and would account for the high count of viable bacterial spores found in the original sample submitted. Further tests have indicated that a minimum of 8 lb. pressure for 12 minutes would be required, and even then the water in the cooker must be brought to the boil before containers are inserted and the cooker closed, if adequate processing is to be assured.

III. The pears canned under these conditions were in each case quite satisfactory, attaining temperatures above that required to inactivate the enzyme system, and gave no signs of browning on opening. Glass jars warmed before filling with pears and boiling syrup, then processed in boiling water, were equally satisfactory, attaining a maximum internal temperature of 201° F. The metals, if any, exposed in the fruit lacquered cans do not

therefore seem to play a part in the browning, provided that the normal canning practice is followed.

IV. The method of processing fruit in an oven, then filling with boiling syrup afterwards, is one which is quite unsuitable for pears. During the long, slow heating before the syrup is added, oxidation of the tannins occurs readily, so that even though inactivation of the enzyme may possibly occur after the syrup is added, the discolouration is already present. Although a similar time is taken to reach maximum temperature in Method I, the fruit is protected by syrup, which prevents browning during processing.

The illustrations in *Plate* V show pears at the C stage of ripeness, photographed the day after opening the containers. The numbers refer to the methods of processing above.

The lighter patches on one of the pears in IV show where this fruit was pressed against the side of the jar during oven processing.

**Enzyme System.**

Other workers (3) (4), have designated the term oxidase to the enzyme system concerned in the browning of apple tissue, and have shown it to consist of a peroxidase and an organic peroxide.

The oxidation of tannin in tea leaves has been summarised in the following manner (5) (6) :—

$$\text{Tea tannin (A)} \underset{\text{dehydrogenase} + 2H}{\overset{\text{oxidase} - 2H}{\rightleftharpoons}} \text{orthoquinone (B)} \longrightarrow \text{condensed tannin (C)}$$

In normally respiring leaves the cyclic reaction A → B → A takes place but damage interferes with B → A and the formation of the pigmented condensed tannin proceeds B → C.

During the investigation of the primarily practical problem with which this paper is concerned, it was observed that the browning of pear tissue was brought about by the conversion of catechol tannin to a brown pigment, an essential step in the process being oxidation of the catechol by an organic peroxide in the presence of peroxidase. When all three were present in pear tissue no pigmentation resulted unless damage occurred, which suggests that the oxidation is normally reversed by a dehydrogenase system as in tea-leaves. When the tissue was damaged, pigmentation occurred at a pace directly proportional to the amount of damage. This was probably due to increased oxygenation by atmospheric oxygen resulting in rapid completion of the reaction A → B → C.

The results of heat-treatment tests on the pear pulp indicate that the oxygen-donating component of the oxidase system, an organic peroxide, is inactivated at 161° F., but the peroxidase activity and an ability to render molecular oxygen available for catechol oxidation remain up to 180-187° F. In pears processed at lower temperatures oxidation would be unable to proceed in the unopened container owing to low oxygen pressure. On opening the container the sudden access of oxygen causes rapid browning. Possibly oxidation to the orthoquinone stage occurs during the early stages of processing before the peroxide is inactivated, so that oxidation is rapidly completed when further oxygen is available.

A " tannase " extract from micro-organisms has been reported (7) and the possibility was examined of the formation of an extra-cellular oxidising enzyme

by the bacteria present in processed pears which showed rapid browning on opening the container. Inoculation tests showed that this was not the case.

## SUMMARY.

1. The tendency of cut pears to darken depends on the catechol tannin content of the fruit. This is not only a varietal character, but varies with the origin or storage methods of the fruit and the degree of ripeness, being greatest at the " firm-ripe " stage.

2. The enzyme system concerned in the browning appears to consist of a peroxidase and an organic peroxide, which together form an oxidase concerned in the oxidation of the catechol tannins.

3. Oxidase activity increases up to about 150° or 160° F., and thereafter lessens, being inactivated between 180° and 187° F. Slight peroxidase activity persists up to 200—210° F. Oxidase, once inactivated by heat, is not regenerated.

4. Quick processing of the fruit, to attain temperatures above the maximum at which enzyme activity can occur, is required to give good results. If this is attained, no browning on exposure to the air after opening will normally occur.

## ACKNOWLEDGMENTS.

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*Plate* V. Browning in pears processed by Methods 1-4.

# RAINFALL AT LONG ASHTON, 1914 TO 1948.

By G. E. CLOTHIER.

The recording of rainfall at Long Ashton Research Station was begun in 1914, and as the conclusion of the present year completes a 35 year period, the opportunity is taken of summarising the records made during that time.

The Research Station lies at an altitude of 160′ above sea level in a shallow valley which widens out to the Bristol Channel ten miles away to the west, and falls away gently towards Bristol some four miles distant to the east. To the north the ground rises sharply to a plateau some 400′ above sea level, but to the south the slope is more gentle and undulating, and in this direction, about ten miles away, are the Mendip Hills, which have a considerable influence on the rainfall of the Station.

Since the meteorological plot was first established, it has been moved twice to avoid the screening effect caused by new buildings erected in the vicinity. The present site is a level piece of ground freely exposed. An 8″ gauge is used, the rim being 12″ above ground level.

Table I gives the rainfall in inches month by month for each of the years 1914 to 1948, together with the means for each month and the totals for each year.

## Annual Rainfall.

The annual normal rainfall for Long Ashton over the standard period 1881 to 1915, as computed by the Meteorological Office of the Air Ministry from the records of other rainfall stations in the district, is 34·13″, which is higher than the general average rainfall for England, 32·67″, for the standard period. The average for the period under review is 36·30″.

The driest year was 1921 and the wettest 1924, the former with a rainfall 31% below and the latter 35% above normal, giving a total fluctuation over the period of 66% of the normal.

An examination of *Fig.* 1 shows that these extremes are unusual and in fact the annual rainfall over the 35 years has only deviated from the normal by +20% in three years and by —20% on the same number of occasions. The graph shows other outstanding features, one of which is a preponderance of wet years during the first half of the period, and of dry years during the second half.

The " record " dry year of 1921 is remarkable as the only really dry year between 1914 and 1932, a period during which the annual rainfall was often well above normal, 1924 being notable as the " record " wet year, and a succession of four wet years occurring from 1927 to 1930. Although 1935 was very wet, the two dry years 1933 and 1934 were the forerunners of a period of thirteen years from 1933 to 1945 during which the annual rainfall was below normal in every year except three, culminating in a spell of five consecutive dry years, 1941 to 1945.

## Monthly Rainfall.

The average values of monthly rainfall have already been given in Table I, and they are also shown in diagrammatic form in *Fig.* 2. It will be seen that there is a well defined annual rhythm from a minimum in the spring to a

TABLE I.

RAINFALL AT LONG ASHTON, 1914-1948.

MONTHLY AND ANNUAL TOTALS (INCHES).

| Year | Jan. | Feb. | Mar. | April | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1914 | 1·47 | 2·99 | 5·10 | 2·03 | 1·99 | 2·53 | 5·33 | 3·19 | 1·85 | 2·07 | 4·03 | 6·25 | 38·83 |
| 1915 | 3·22 | 4·08 | 0·96 | 1·24 | 3·96 | 0·97 | 5·21 | 2·26 | 1·77 | 4·33 | 1·97 | 6·59 | 36·56 |
| 1916 | 2·89 | 4·89 | 3·57 | 1·29 | 4·24 | 2·81 | 1·30 | 5·74 | 2·01 | **7·68** | 3·17 | 4·34 | 43·93 |
| 1917 | 1·14 | 1·50 | 3·49 | 2·35 | 2·22 | 2·83 | 2·28 | **8·16** | 2·86 | 6·84 | 0·78 | **0·49** | 34·94 |
| 1918 | 3·79 | 2·19 | 1·56 | 2·59 | 1·53 | 1·40 | 4·22 | 2·64 | **9·79** | 2·36 | 2·31 | 5·09 | 39·47 |
| 1919 | 4·94 | 3·07 | **7·57** | 2·61 | **0·36** | 1·67 | 2·88 | 3·17 | 1·81 | 3·21 | 2·87 | 7·96 | 42·12 |
| 1920 | 4·65 | 1·99 | 2·67 | 4·38 | 1·85 | 3·44 | 5·19 | 1·86 | 2·09 | 2·40 | 2·05 | 3·16 | 35·73 |
| 1921 | 3·67 | 0·27 | 2·18 | 1·03 | 2·12 | 0·24 | **1·02** | 3·07 | 4·89 | 1·68 | 2·37 | 2·66 | **25·20** |
| 1922 | 3·23 | 4·54 | 3·45 | 3·19 | 1·05 | 1·89 | 4·47 | 4·04 | 2·67 | 1·69 | 1·66 | 5·09 | 36·97 |
| 1923 | 1·92 | **7·12** | 1·34 | 2·70 | 1·88 | 0·51 | 2·35 | 3·50 | 2·94 | 4·77 | 3·19 | 2·75 | 34·97 |
| 1924 | 4·63 | 0·68 | 1·80 | 2·98 | 4·32 | **4·45** | 5·01 | 5·79 | 3·64 | 6·38 | 3·27 | 6·12 | **49·07** |
| 1925 | 3·27 | 4·65 | 0·60 | 1·79 | 4·90 | **0·03** | 3·45 | 3·00 | 3·28 | 3·74 | 2·38 | 4·55 | 35·64 |
| 1926 | 5·06 | 1·91 | 0·68 | 2·91 | 2·83 | 1·82 | 2·29 | 2·65 | 2·02 | 5·16 | 8·02 | 0·56 | 35·91 |
| 1927 | 3·33 | 3·41 | 3·01 | 1·49 | 1·13 | 3·45 | 5·27 | 4·41 | 6·65 | 2·87 | 3·21 | 3·27 | 41·50 |
| 1928 | 6·28 | 3·69 | 2·86 | 1·39 | 1·11 | 2·87 | 3·17 | 3·15 | 1·18 | 7·26 | 3·76 | 3·75 | 40·47 |
| 1929 | 1·15 | 1·41 | 0·55 | 0·90 | 2·48 | 2·22 | 1·84 | 3·88 | 0·74 | 6·76 | **9·92** | **8·08** | 39·93 |
| 1930 | 5·33 | 0·55 | 2·22 | 2·70 | 1·45 | 1·98 | 2·38 | 5·17 | 4·24 | 3·89 | 4·82 | 3·73 | 38·46 |
| 1931 | 2·25 | 2·27 | 0·86 | 2·72 | 3·47 | 3·50 | 4·45 | 3·96 | 2·95 | 1·12 | 4·69 | 2·23 | 34·47 |
| 1932 | 4·53 | **0·05** | 2·60 | 3·56 | **5·93** | 1·42 | 2·72 | 1·21 | 5·82 | 7·48 | 2·02 | 1·60 | 38·94 |
| 1933 | 3·56 | 3·93 | 3·71 | 1·18 | 2·22 | 1·19 | 1·96 | 1·41 | 2·34 | 2·84 | 0·57 | 1·43 | 26·34 |
| 1934 | 2·16 | 0·41 | 2·21 | 2·79 | 1·46 | 1·56 | 1·70 | 3·08 | 2·55 | 2·21 | 1·32 | 7·13 | 28·58 |
| 1935 | **1·07** | 3·24 | 0·68 | **4·85** | 1·11 | 4·20 | 2·04 | 1·97 | 7·88 | 6·34 | 6·19 | 4·41 | 43·98 |
| 1936 | 3·53 | 2·65 | 2·51 | 2·71 | 1·13 | 2·93 | 5·85 | 0·56 | 3·18 | 1·93 | 4·78 | 3·47 | 35·23 |
| 1937 | 4·43 | 5·16 | 3·48 | 2·62 | 2·56 | 1·41 | 3·99 | 2·01 | 2·11 | 3·32 | 2·05 | 2·10 | 35·24 |
| 1938 | 4·90 | 1·11 | **0·46** | **0·25** | 2·19 | 1·29 | 3·94 | 3·36 | 2·28 | 5·92 | 5·18 | 3·57 | 34·45 |
| 1939 | 5·76 | 2·28 | 1·67 | 3·27 | 0·89 | 2·11 | 4·46 | 3·07 | 1·04 | 2·93 | 7·06 | 2·34 | 36·88 |
| 1940 | 2·44 | 2·29 | 2·65 | 2·20 | 2·68 | 1·08 | **7·92** | **0·19** | 1·91 | 4·34 | 8·65 | 1·60 | 37·95 |
| 1941 | 3·49 | 3·42 | 3·37 | 1·27 | 1·71 | 2·20 | 2·32 | 4·82 | **0·50** | 2·18 | 1·82 | 2·65 | 29·75 |
| 1942 | 3·71 | 0·17 | 2·08 | 1·21 | 5·12 | 0·40 | 2·91 | 4·71 | 2·00 | 4·12 | 1·52 | 3·91 | 31·86 |
| 1943 | 6·06 | 1·98 | 1·25 | 1·30 | 2·84 | 1·89 | 1·96 | 4·26 | 2·89 | 3·08 | 2·21 | 2·08 | 31·80 |
| 1944 | 2·06 | 0·79 | 0·47 | 2·88 | 1·20 | 3·18 | 2·11 | 3·55 | 3·93 | 4·81 | 6·36 | 2·83 | 34·17 |
| 1945 | 2·16 | 3·75 | 1·16 | 1·02 | 3·56 | 3·61 | 1·88 | 2·65 | 2·21 | 3·94 | **0·53** | 5·02 | 31·49 |
| 1946 | 3·55 | 2·94 | 1·15 | 2·26 | 4·14 | 3·45 | 1·62 | 7·41 | 4·46 | 1·05 | 4·94 | 3·43 | 40·40 |
| 1947 | 2·42 | 1·61 | 6·30 | 2·77 | 3·07 | 1·74 | 3·28 | 0·84 | 2·01 | **0·55** | 2·68 | 3·08 | 30·35 |
| 1948 | **6·37** | 1·97 | 1·69 | 2·22 | 3·67 | 2·59 | 1·34 | 4·69 | 2·79 | 4·40 | 2·02 | 5·30 | 39·05 |
| Average | 3·55 | 2·54 | 2·34 | 2·25 | 2·52 | 2·14 | 3·26 | 3·41 | 3·07 | 3·88 | 3·55 | 3·79 | 36·30 |

Figures in bold type indicate the largest and smallest monthly and annual totals.

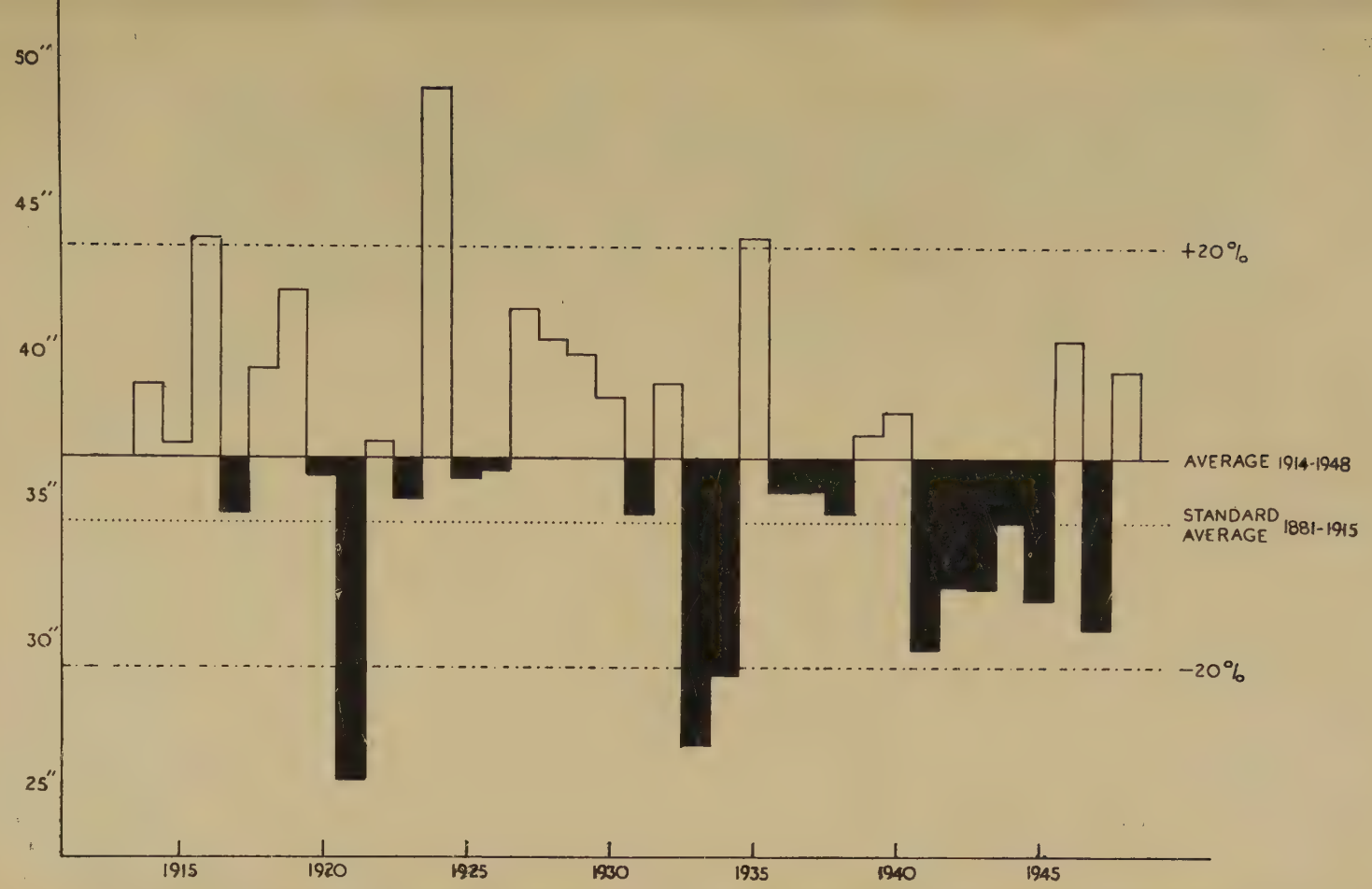


*Fig.* 1. Fluctuations in Annual Rainfall, Long Ashton, 1914 to 1948. Unshaded columns indicate deviations above the average. Shaded columns indicate deviations below the average.

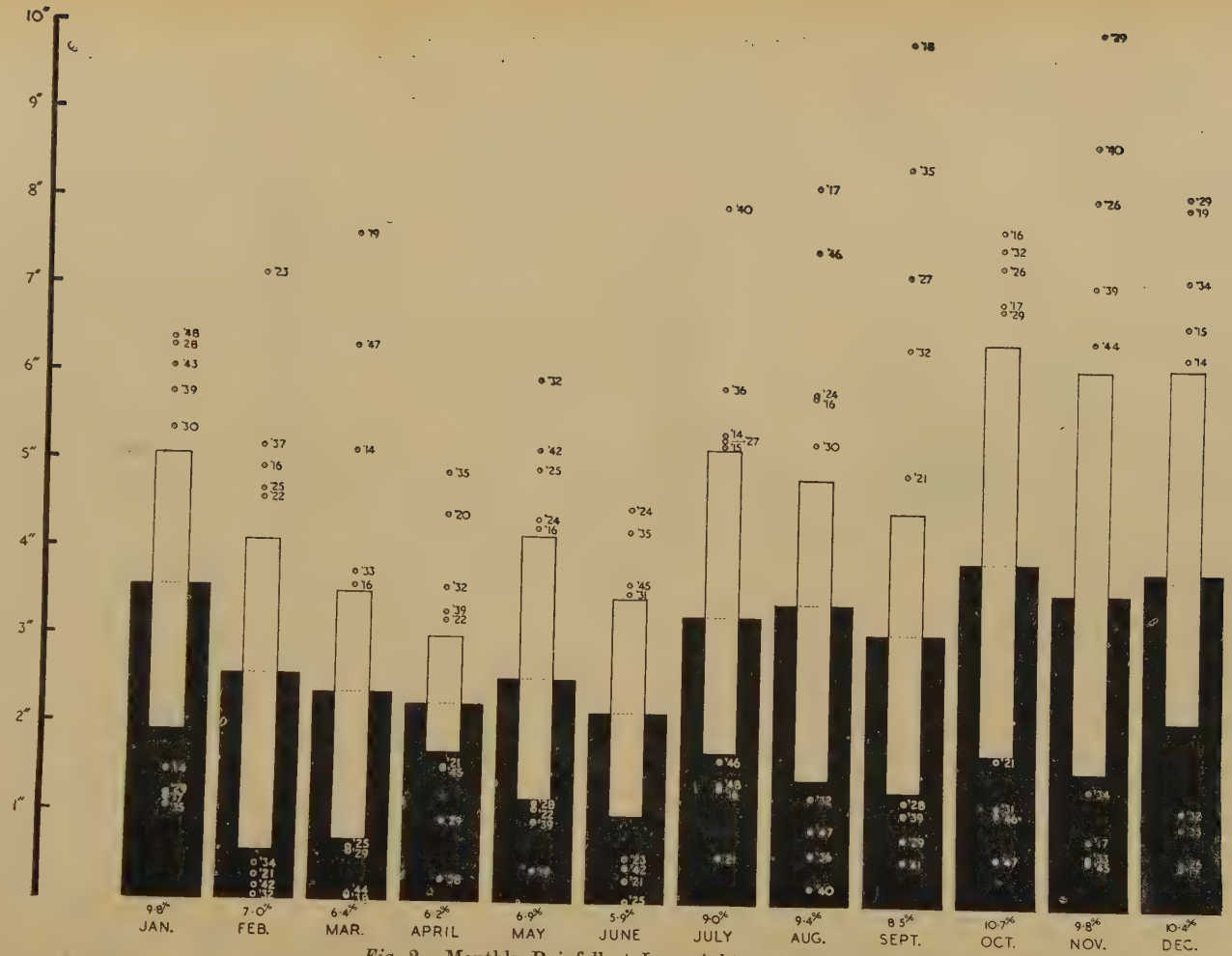


*Fig.* 2. Monthly Rainfall at Long Ashton, 1914-1948.

Black columns indicate mean monthly rainfall. Superposed white columns indicate the range of 80% of the records. Totals above and below the 80% range and the years in which they were recorded, are also indicated.

winter maximum. From December to June, the driest month, there is a steady decrease, sharp from January to February (partly accounted for by the fewer days in February), and only broken by a slight increase in May. A sharp rise occurs in July, the curve rising to a maximum in October, the wettest month, and then falling slightly to December. The continuity of the curve is broken by a decrease in September, and, to a lesser extent, in November.

**Seasonal Rainfall.**

The distribution of rainfall throughout the year, expressed in percentages of the normal total, is: January–March 23%, April—June 19%, July—September 27%, October—December 31%.

January to March is shown by the averages to be a well marked period of change from wet to dry, but it is occasionally continuously wet, as in 1919, or dry, as in 1929. Long periods with little rain are not uncommon in the late winter and early spring months. Their onset is seldom before mid or late January, though there was a well marked exception during the winter of 1939—1940, when from December 10th 1939 to January 24th 1940, only 0·55″ of rain was recorded. Dry periods early in the year are often accompanied by persistent N.E. and E. winds. A good example of this type of weather was to be found in 1932, when from January 18th to March 25th only 0·70″ was recorded, 0·40″ of this total being collected during a temporary break in the weather early in March.

In 1929, a year in which the monthly distribution of rainfall was unprecedented throughout the British Isles, the total fall in January was 1.15″, of which 0·78″ was accounted for in two consecutive days. Only 0·73″ fell between February 3rd and March 19th, and of this total 0·42″ was recorded in one day. In the famous dry year of 1921, February's total of 0·27″ was collected in two consecutive days.

In more recent years, 1943 and 1948 will be remembered for dry periods in early spring, the former yielding a total of 0·25″ between February 9th and March 22nd and the latter 0·09″ from February 15th to March 14th.

Out of a total of 25 " rain spells " (i.e. a period of at least 15 days, each of which is credited with 0·01″ or more of rain) recorded at Long Ashton, nine have covered parts of January. The wettest January was that of 1948, with a total of 6·37″, and the wettest February was in 1923 with a total of 7·12″, an outstandingly wet month. The monthly total for March has only exceeded 4·0″ on three occasions, but the largest total of 7·57″ in March 1919 marks the wettest month of any at this period of the year.

Amounts of less than one inch have not been recorded in January, but have occurred on seven occasions in February, and eight in March, the smallest total being that of February 1932 with 0·05″.

In the late spring and early summer months there have been some outstanding dry periods. 1915 provided a rainless spell of 31 days from May 22nd until June 21st, an " absolute drought " (i.e. a period of at least 15 consecutive days, to none of which is credited as much as 0·01″ of rain). In 1925, June's total of 0·03″ was recorded on the first day of the month, this being the driest month of any since records commenced at Long Ashton. In 1939, following small amounts of rain in the first part of May, a rainless spell ensued from the 22nd until June 9th.

The sharp increase in the average rainfall which is shown to take place

in July is often anticipated in June, the record of " rain spells " showing three commencing in mid or late June.

The outstanding wet July is that of 1940, with a total of 7·92″, well above the next wettest in 1936 with 5·85″. A total of 5″ has been exceeded seven times, mostly in the early years, while it is interesting to note that in seven of the past eight years the July total has been below normal. August rainfall varies from two outstandingly wet months in 1917 and 1946, with 8·16″ and 7·41″ respectively, to the three years 1936, 1940 and 1947 when the totals were less than 1·00″. In 1947, " absolute drought " prevailed from August 5th to September 6th, 33 days. The lowest total of 0·19″ in 1940 followed the very wet July. September rainfall shows less deviation from the normal than that of the two previous months, and in 20 years out of the 35 the total lies between 0·75″ and 3·00″, which makes the total of 9·79″ in 1918 all the more remarkable, this being the second wettest month in the records.

The rainfall in the three months July to September in 1918 amounted to 16·65″, and was almost equalled by the figure of 16·33″ for the same period in 1927, another year with a wet September.

The most marked dry spells in this season have occurred in more recent years. In 1935 from July 2nd to August 23rd only 0·42″ was recorded, except on July 11th, when 1·52″ was collected as the result of thunderstorms. Also, in 1937 little more than 0·50″ was measured from July 16th to September 8th, except for 1·71″ during two consecutive thundery days about the middle of the period. The records of these two years illustrate the fact that the development of thunderstorms during a dry period in mid summer is not always followed by a complete break in the weather. In both 1940 and 1941 dry periods occurred ; in the former year 0·35″ fell from July 28th to September 11th, and in the latter from August 30th to September 30th the total was 0·50″, collected on three dates, one of which yielded 0·32″.

The months October to December constitute the wettest season, with October the wettest month of the year. Nearly 50% of the recorded " rain spells " have their commencement during this period. Though October is the wettest month on average, the highest total for this month, 7·68″ in 1916, has been exceeded three times in November and twice in December. November, with an average slightly less than that of the other two months, provides the wettest month of any on record with 9·92″ in 1929. In that year, these three months provided a spell remarkable for wetness in many parts of Britain, and at Long Ashton the total for the three months was 24·76″, or 62% of the years total. One " rain spell " of 24 days, from November 22nd to December 15th, yielded 10·40″, and was closely followed by another of 28 days from December 19th with a total of 6·27″.

At the other end of the scale, the lowest total for these three months was 4·84″ in 1933, during which there was a " dry spell " of fifteen days from October 28th to November 12th with a total rainfall of only 0·07″.

**Heavy Falls in a Day.**

Heavy falls in a day are fortunately uncommon at Long Ashton, the total having exceeded 2·00″ on only three occasions :—November 2nd 1940 with 2·35″, October 5th 1929 with 2·32″ and the same amount on July 15th 1937.

Amounts of over one inch in a day have been recorded on 76 occasions, distributed over the months as follows :—October 13, November 10, May 9, September 9, August 9, July 8, December 6, January 4, June 4, February 2, March 1, April 1.

## SUMMARY.

1. Records of the monthly and annual rainfall at Long Ashton during the years 1914 to 1918 are given.

2. During this period, the annual fall has varied between 49·07″ and 25·20″, the mean figure being 36·30″. The first half of the period shows a preponderance of wet years and the second half of dry years.

3. It is shown that the wettest month, on the average, is October, while the driest is June. For every month in the year there are many instances of totals which are much higher or lower than the mean total for the month.

4. On the average, the rainfall for the second half of the year is distinctly greater than that of the first half. The period October to December is the wettest quarter and April to June is the driest.

5. During the last 35 years daily falls of more than 2·0″ have occurred on three occasions and amounts of over 1·0″ in a day have been recorded 76 times.

# METEOROLOGICAL RECORDS, 1948.

| Month | Air Temperature | | | | | | | Rainfall | | | Sunshine | | | Soil Temperature at 09.00 hours | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | | Mean of A & B | Dif. from average | Absolute Max. | Absolute Min. | Number of Ground Frosts | Total | Dif. from average | Most in a day | Total hours | Dif. from normal | Daily average | 4″ | 8″ | 24″ |
| | Max. (A) | Min. (B) | | | | | | | | | | | | | | |
| January | 49·0 | 37·4 | 43·2 | +2·6 | 57 | 28 | 14 | 6·37 | +2·82 | 0·99 | 38·7 | — 9·5 | 1·25 | 40·7 | 41·8 | 43·6 |
| February | 46·7 | 36·2 | 41·5 | +0·7 | 62 | 21 | 15 | 1·97 | —0·57 | 0·55 | 84·9 | +17·7 | 2·93 | 39·8 | 39·2 | 43·0 |
| March | 57·5 | 38·4 | 48·0 | +4·3 | 70 | 30 | 15 | 1·69 | —0·65 | 0·62 | 170·3 | +47·8 | 5·49 | 43·5 | 44·5 | 45·3 |
| April | 57·3 | 39·1 | 48·2 | +0·6 | 69 | 31 | 11 | 2·22 | —0·03 | 0·59 | 211·4 | +56·3 | 7·05 | 46·9 | 47·0 | 49·1 |
| May | 63·4 | 43·9 | 53·7 | +1·5 | 78 | 32 | 5 | 3·67 | +1·15 | 1·04 | 286·3 | +94·7 | 9·24 | 55·4 | 54·6 | 55·6 |
| June | 63·7 | 50·5 | 57·1 | —1·4 | 73 | 45 | 0 | 2·59 | +0·45 | 0·79 | 156·9 | —56·9 | 5·23 | 57·6 | 57·4 | 58·4 |
| July | 69·0 | 52·6 | 60·8 | —1·6 | 90 | 41 | 0 | 1·34 | —1·92 | 0·44 | 183·0 | —11·1 | 5·90 | 62·0 | 61·7 | 61·0 |
| August | 66·9 | 53·1 | 60·0 | —1·1 | 72 | 44 | 0 | 4·69 | +1·28 | 1·75 | 155·4 | —28·5 | 5·01 | 60·1 | 60·2 | 62·3 |
| September | 65·1 | 50·8 | 57·9 | +0·8 | 73 | 33 | 1 | 2·79 | —0·28 | 0·97 | 160·9 | +17·6 | 5·36 | 57·1 | 57·5 | 60·3 |
| October | 57·4 | 44·2 | 50·8 | —0·2 | 68 | 28 | 6 | 4·40 | +0·52 | 1·02 | 98·5 | — 2·9 | 3·18 | 50·3 | 51·6 | 55·8 |
| November | 53·7 | 40·8 | 47·3 | +3·0 | 64 | 26 | 9 | 2·02 | —1·53 | 0·44 | 73·1 | +16·8 | 2·44 | 45·0 | 46·0 | 49·5 |
| December | 49·2 | 37·5 | 43·2 | +2·0 | 58 | 18 | 14 | 5·30 | +1·51 | 0·68 | 61·9 | +18·1 | 1·96 | 41·8 | 42·7 | 46·1 |
| Total or Mean | | | 50·9 | +0·9 | | | 90 | 39·05 | +2·75 | | 1681·3 | +160·1 | 4·59 | | | |

G. E. Clothier.

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